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COVER

Pronghorn, *Antilocapra americana*

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WATER AND NUTRIENT CONTENT OF FORAGE IN SONORAN PRONGHORN HABITAT, ARIZONA

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We examined diets of Sonoran pronghorn, *Antilocapra americana sonoriensis*, to determine if they could meet their total water requirements with and without selective foraging. Water availability was based on seasonal measures of plant species composition, moisture, and nutrient content at 2 sites within Sonoran pronghorn range in southwestern Arizona from June 1995 to May 1996. We measured vegetation forage availability using the dry-weight-rank and comparative yield methods and preformed water and organic nutrient content of vegetation using standard laboratory procedures. Water and nutrient content of plants varied significantly between the 2 sites and among 5 seasons. Plant species consumed by pronghorn were higher in moisture and selected nutrients than those not used for forage. Daily maximum water intake predicted by diet models ranged from 1.1 to 6.1 liters/animal/day. After subtracting water theoretically required for excretion, respiration, and evaporation (approximately 50%), predicted water intake from forages was not adequate to meet minimum water requirements (1.8–3.4 liters/animal/day) for 14 of 20 simulated diets. Water developments may improve Sonoran pronghorn habitat if water is a limiting factor for the population and water requirements cannot be met by forage consumption.

INTRODUCTION

Sonoran pronghorn, *Antilocapra americana sonoriensis,* inhabit the Sonoran Desert in southwestern Arizona and western Sonora, Mexico (Goldman 1945). In 1967, Sonoran pronghorn were listed as an endangered subspecies by the United States Fish and Wildlife Service (USFWS). Although other pronghorn subspecies inhabiting the western United States (i.e., American pronghorn, *A. a. americana;* Mexican pronghorn, *A. a. mexicana*; and Oregon pronghorn, *A. a. oregona*) have partially recovered from population declines in the early 1900s (Einarsen 1948; Yoakum 1986, 1994), the peninsular pronghorn, *A. a. peninsularis*, of Baja

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California, and the Sonoran pronghorn (Yoakum et al. 1996) have not. In 1994, there were only 250 Sonoran pronghorn in Arizona and 310 in Mexico (Thompson-Olais[3] 1994).

Historically, more than 700 Sonoran pronghorn ranged from eastern California to central Arizona and central Sonora, Mexico and from Hermosillo, Mexico north to the Altar Valley and the Tohono O'Odham Reservation in Arizona (Nelson[4] 1925, Wright and deVos[5] 1986). At present, Sonoran pronghorn range from near Yuma, Arizona; east to central Arizona; south to near Caborca, Mexico; and west to the Gulf of California (Wright and deVos[5] 1986, Yoakum et al. 1996). Range constriction and past population declines of Sonoran pronghorn may be related to the elimination (i.e., diversion) of perennial water sources (Wright and deVos[5] 1986). All perennial free-standing water now available to Sonoran pronghorn is from a few anthropogenic water catchments. To adequately determine if water is a limiting factor for Sonoran pronghorn, managers must determine whether pronghorn can obtain enough water from forage plants in their diets or if water catchments are necessary to supplement pronghorn water intake.

American pronghorn diet and nutritional content of forage species have been well documented (Severson et al. 1968[6], Schwartz and Nagy 1976, Roebuck et al. 1982), but such information is not available for Sonoran pronghorn. Plant species lists for Sonoran pronghorn diet have been compiled from fecal analysis (Carr[7,8] 1970, 1972; AGFD[9] 1981; Hughes[10] 1991), but attributes of forage species and habitat requirements for Sonoran pronghorn remain undocumented. Effective management depends on understanding habitat requirements for Sonoran pronghorn (O'Gara and Yoakum 1990, Cook et al. 1996, Yoakum et al. 1996). Our objectives were to assess water content and nutrient content of forages within Sonoran pronghorn range,

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[3] Thompson-Olais, L.A. 1994. Sonoran pronghorn recovery plan revision. U.S. Fish and Wildlife Service, Region 2, Albuquerque, New Mexico, USA.

[4] Nelson, F.W. 1925. Status of pronghorn antelope, 1922–1924. U.S. Department of Agriculture Bulletin 1346, Washington, D.C., USA.

[5] Wright, R.L. and J.C. deVos Jr. 1986. Final report on Sonoran pronghorn status in Arizona. Arizona Game and Fish Department, Phoenix, Arizona, USA.

[6] Severson, K.E., M. May, and W.G. Hepworth. 1968. Food preferences, carrying capacities, and forage competition between antelope and domestic sheep in Wyoming's Red Desert. Agriculture Experiment Station, Science Monograph 10, University of Wyoming, Laramie, Wyoming, USA.

[7] Carr, J.N. 1972. Endangered species investigation: Sonoran pronghorn. Arizona Game and Fish Department, Phoenix, Arizona, USA.

[8] Carr, J.N. 1970. Endangered species investigation: Sonoran pronghorn. Federal Aid in Wildlife Restoration Program Report Project W-53-R-20, Arizona Game and Fish Department, Phoenix, Arizona, USA.

[9] AGFD. 1981. The Sonoran pronghorn. Federal Aid in Wildlife Restoration Project W-53-R, Special Report 10, Arizona Game and Fish Department, Phoenix, Arizona, USA.

[10] Hughes, K.S. 1991. Sonoran pronghorn use of habitat in southwest Arizona. M.S. Thesis, The University of Arizona, Tucson, Arizona, USA.

and to determine if pronghorn can meet their daily water requirements solely from consumption of forages without the use of free-standing water.

STUDY AREA

Growler Valley, in southern Arizona, is the core of Sonoran pronghorn range in the United States (Thompson-Olais[3] 1994). We selected 2 study areas approximately 75 km apart, based on distribution of pronghorn, availability of vegetation, access to the sites, and permission to use the areas. The Agua Dulce study site was located in the Agua Dulce Mountains at the southern end of Growler Valley (Fig. 1), on the Cabeza Prieta National Wildlife Refuge (CPNWR). The Charlie Bell site was located 75 km north of Agua Dulce, on the bajada (a long outwash slope at the base of a mountain range) west of the Growler Mountains. Both sites were approximately 20 km^2 (Fig. 1), but the Agua Dulce study site was slightly more mesic than the Charlie Bell site (T.L. Cutler, The University of Arizona, personal communication).

Growler Valley is composed of broad alluvial valleys interspersed with steep, block-faulted mountain ranges. Elevations range from 100 to 1,200 m. Mean annual precipitation is 13 cm and occurs primarily from July to September and December to February (Brown 1994). Average temperatures range from 19 to 32°C annually and maximum temperatures often approach 50°C during July and August (Sellers and Hill 1974).

Study sites were composed of the mixed scrub series of the lower Colorado River Valley and Arizona Upland subdivisions of the Sonoran desert scrub vegetation community (Brown 1994). The mixed shrub series of the lower Colorado subdivision was characterized by creosote bush, *Larrea tridentata* and white, *Ambrosia dumosa,* or triangle-leaf, *A. deltoidea*, bursage on flats (i.e., <5% slope) interspersed with ironwood, *Olneya tesota*; blue paloverde, *Cercidium floridum*; and mesquite, *Prosopis* spp., along washes (i.e., ephemeral streams) and around playas (Brown 1994). The Arizona Upland subdivision was composed primarily of blue paloverde; foothill paloverde, *C. microphyllum*; ironwood; mesquite; catclaw acacia, *Acacia greggii*; ocotillo, *Fouquieria splendens*; creosote bush; white and triangle-leaf bursage; limberbush, *Jatropha cuneata*; range ratany, *Kramaria parvifolia*; and desert lavender, *Hyptis emoryi*. Plant nomenclature follows Lehr (1978).

METHODS

We sampled the abundance of individual plant species on 600 (200 in flats and 400 in washes) 0.1-m^2 plots (Daubenmire 1959, Higgins et al. 1994) placed at 10-m intervals along 500-m line transects during 5 seasons (June–July, August–September, October–November, February–March, April–May) from June 1995 to May 1996. Sampling was prohibited during December and January due to federal land closures. For each sampling period, we determined transect origins from a grid of each study area using a random numbers table. Direction of each transect was established by spinning a compass dial. Measurements for each plot included ocular estimates of total herbage yield and species composition using the comparative yield (Haydock

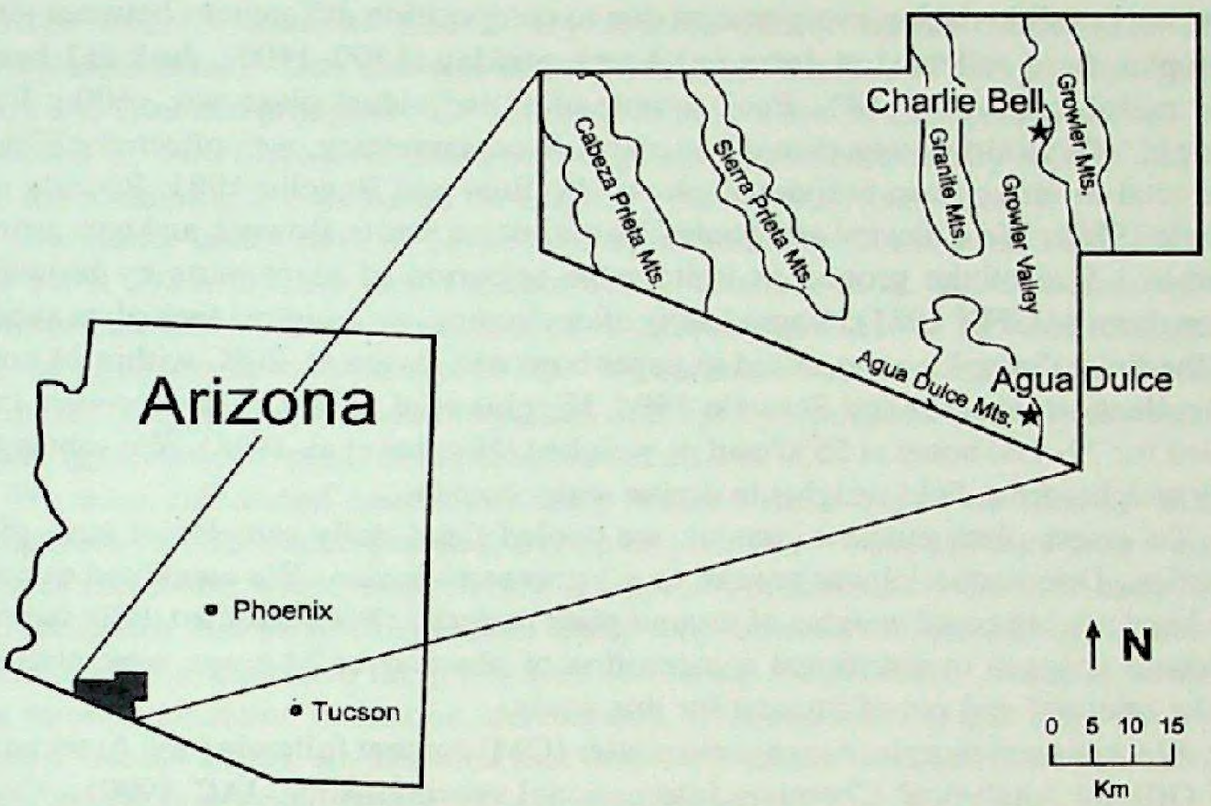

Figure 1. Location of 2 study sites within the Cabeza Prieta National Wildlife Refuge, Pima County Arizona, where water content and the nutrient value of forage were evaluated for Sonoran pronghorn, 1995–1996.

and Shaw 1975) and dry-weight-ranking methods ('t Mannetje and Haydock 1963, Friedel et al. 1988). We used these methods to estimate plant species abundance because they were quicker and easier than using exact percentages and have been documented as appropriate for sampling desert vegetation (Ruyle 1990, Mazaika and Krausman 1991, Mazaika et al. 1992). We harvested 30 1-m^2 plots for double sampling calibrations (Higgins et al. 1994) per season at each study site (1 harvested/20 ranked). Vegetation from all harvested plots was weighed to the nearest 0.1 g, dried at 55°C for $\leq$4 days, and re-weighed on a digital electronic scale. We calculated species biomass (kg/ha dry weight) and total biomass for creosote flats and washes and for each site and season following 't Mannetje and Haydock (1963).

We collected and analyzed samples of forage and non-forage species for water and nutrient content. Plants eaten by pronghorn and selected for analysis were determined from previous diet studies of Sonoran pronghorn (AGFD[9] 1981, Hughes and Smith[11] 1990, Hughes[10] 1991) and the present availability of the plants. Forages collected over the year tracked seasonal changes in pronghorn diets (Yoakum 1990).

To assess plant moisture content, we clipped samples of all forage and non-forage plants available at both study sites during the 5 sampling periods. Samples were collected 4 times over 24 hours for all plant species at both study sites. When possible, identical sets of species were collected at both study sites; however, this

[11] Hughes, K.S. and N.S. Smith. 1990. Sonoran pronghorn use of habitat in southwest Arizona. Project No. 14-16-009-1564-RWO 6, Final Report. Arizona Cooperative Fish and Wildlife Research Unit, Tucson, Arizona, USA.

was not possible during every season due to composition differences between sites. Samples were collected at dawn (±1 hour), midday (1300–1500), dusk (±1 hour), and midnight (1100–0100). Each sample of an individual plant was ≤600 g fresh weight. To minimize our damage to plants from harvesting, we collected <30% of the total biomass from individual plants (Wallmo and Regelin 1981, Roundy and Ruyle 1989). We collected and pooled leaves, stems, fruits, flowers, and new growth within 1.5 m of the ground to imitate the selection of plant parts by browsing pronghorn (AGFD[9] 1981). Immediately after clipping, we weighed each plant sample in the field. Samples were stored in paper bags and frozen at -20°C within 24 hours of collection (Haufler and Servello 1994, Higgins et al. 1994). Samples were later dried for 72–120 hours at 55°C and re-weighed (Higgins et al. 1994). We subtracted dry weights from field weights to derive water content.

To assess plant nutrient content, we pooled the 4 daily samples of each plant species. Dried material was ground to a 2-mm particle size. We combined samples by hand mixing equal weights of ground plant material. We combined daily samples because changes in nutritional composition of plants over 24 hours were assumed to be minimal and not of interest for this study.

We analyzed samples for organic matter (OM) content following the Association of Official Analytical Chemists International procedures (AOAC 1990). Crude protein (CP) was measured with a N Auto Analyzer (Technicon[12], Tarrytown, New York, USA) following micro-kjeldahl digestion (AOAC 1990), lipid content by chloroform-methanol extraction (AOAC 1990), acid detergent fiber (ADF: cellulose, lignin, and silica) following Goering and Van Soest (1970), and neutral detergent fiber (NDF: cellulose, hemicellulose, lignin, and insoluble ash) following Robertson and Van Soest (1981). We determined ash content by incinerating 2 g of dried plant sample at 500°C for 24 hours and weighing the residue. We calculated nitrogen free extract (NFE) as the dry matter not accounted for by the sum of crude protein, lipid extract, crude fiber, and ash.

We used Bartlett's test for homogeneity of variances and visual inspection of normal probability plots to examine data sets for equal variances and departures from normality (Sokal and Rohlf 1995). We compared water and nutrient content of forage and non-forage plants among sites and seasons using 1-way or 2-way ANOVA and Student-Newman-Keuls multiple range tests or Bonferroni tests. When appropriate, we restricted comparisons made using 2-way ANOVAs to specific sites and seasons. In selected cases, we used Student's t-tests to compare group means between sites. To more closely meet the assumption of normality, all analyses were performed on arcsine-transformed data at $P \leq 0.05$ or at a group alpha level appropriate for testing multiple comparisons at $P \leq 0.05$. We pooled nutrient values of forage and non-forage species across sites or seasons to estimate dietary intake when no significant differences existed.

We calculated forage consumption for 5 pronghorn diets using field data from

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[12] Use of trade names does not imply endorsement by the California Department of Fish and Game.

2 sites over 5 seasons and 2 dry matter (DM) intake rates (1.3 and 1.5 kg/animal/day). One diet was based on relative biomass of forages during this study and assumed pronghorn used forages in proportion to their presence (i.e., "no selection"). Two diets were based on forage selection described by AGFD[9] (1981) and the remaining 2 were based on Hughes[10] (1991) (i.e., "selection").

Dry matter intake rates of 1.3 and 1.5 kg/animal/day (Knox et al. 1969 , Hobbs and Swift 1985, O'Gara and Yoakum 1992, Yoakum et al. 1996) were used to represent the daily food requirements of an adult female (31–48 kg) and adult male (41–64 kg) pronghorn (Yoakum 1978). We estimated nutrient intake for each diet by multiplying the protein, carbohydrate, and lipid proportions by daily dry matter intake (g) (Schwartz et al. 1977, Goodson et al. 1991).

We also calculated maximum daily water intake based on these 5 diets. Anhydrous carbohydrates, proteins, and lipids yield 56, 40, and 107% of their weight in total metabolic water, respectively (Houpt 1970, Robbins 1993) for each diet. We recognize that these calculations likely overestimate the amount of metabolic water readily available to the pronghorn following metabolism. Because water is lost through excretion (urination, defecation), from respiration, and from the body surface (Houpt 1970), we assumed that only 50% of the total calculated metabolic water production would be available to the animal.

Empirical data describing the water requirements of Sonoran pronghorn were unavailable. To determine the daily amount of water theoretically required by these animals we used water turnover requirements (Y) from the following allometric relationship for free-ranging, eutherian herbivores: $Y(ml) = 0.71W\ (g)^{0.80}$ (Robbins 1993). Water requirements predicted by Richmond et al. (1962) were 1.8 liters/29 kg animal/day and 3.4 liters/64 kg animal/day based on diets containing 1.3 kg/animal/day and 1.5 kg/animal/day dry matter intake, respectively. We compared our water intake estimates (i.e., preformed and metabolic) from diet models (Appendix 1) to these water requirements.

RESULTS

Vegetation Abundance

Estimates for total standing crop on creosote flats at Agua Dulce were 659.7 kg/ha in February–March, 548.0 kg/ha in April–May, 418.8 kg/ha in June–July, 736.8 kg/ha in August–September, and 452.6 kg/ha in October–November. Estimates for washes at Agua Dulce for the same 5 seasons were 1,216.5, 1,058.8, 884.1, 1,228.5, and 979.5 kg/ha, respectively. Estimates for total vegetation biomass (dry weight) on creosote flats at Charlie Bell were 541.4 kg/ha in February–March, 496.5 kg/ha in April–May, 462.9 kg/ha in June–July, 497.7 kg/ha in August–September, and 563.5 kg/ha in October–November. Estimates for washes at Charlie Bell for the same 5 seasons were 834.2, 684.8, 718.6, 793.0, and 929.3 kg/ha, respectively. Standing crop at the 2 study sites across the 5 seasons varied from 0 to 250 kg/ha for individual plant species. The mean weights for harvested plots ranked 1–5 (Fox[13] 1997) met the assumption of linearity required for the use of

the comparative yield method (Haydock and Shaw 1975).

Numbers of plant species sampled at Agua Dulce for water and nutrient content were: 12 during February–March, 11 in April–May, 10 in June–July, 12 in August–September, and 12 in October–November. Numbers of plant species sampled at Charlie Bell for water and nutrient content were: 9, 9, 9, 9, and 10 in the above months. Plants sampled at these 2 sites included a variety of 15 species for analysis each season.

Plant Water Content

Percent moisture data for individual plant species (Fox[13] 1997) were pooled for each 24-hour sampling period because no significant differences were detected for samples collected among time periods ($n = 4$, $P > 0.05$) at either site or for any season. Plant moisture for all seasons was significantly higher at Agua Dulce (50.2%) than at Charlie Bell (38.9%) ($P < 0.001$). The highest overall (forage and non-forage) plant moisture occurred at Agua Dulce in October–November (58.2%) and during April–May (44.9%) at Charlie Bell. The lowest overall plant moisture occurred at Agua Dulce in April-May (43.1%) and during October–November (33.2%) at Charlie Bell. Significant site x season ($P < 0.001$) and season x forage type ($P = 0.004$) interactions existed. Therefore, we assessed seasonal differences in plant moisture for each study site and forage type individually.

Significant differences in water content among plants used as forage by pronghorn occurred among 5 seasons for both study sites (1 and 2-way ANOVA, $P < 0.001$) (Table 1). At Agua Dulce, mean percent moisture content for forage species (all seasons) was significantly higher than non-forage species ($P = 0.011$). At Charlie Bell, mean percent moisture for forage and non-forage plant species (all seasons) was not significantly different ($P = 0.234$).

Plant Nutrient Content

Nutrient content for plant species did not differ significantly among seasons at either site ($P > 0.05$). We pooled seasonal values for crude protein, NFE, lipid, NDF, ADF, and ash content and assessed differences between sites and forage type using t-tests for all pair-wise comparisons.

Crude protein and lipid content of forage plants did not differ significantly between sites (Table 2). The NFE and ash content were higher in forages at Agua Dulce (30.3 and 8.7%, respectively) than at Charlie Bell (26.1 and 7.1%, respectively). The values for NDF and ADF were also approximately 10 to 15% higher at Charlie Bell than at Agua Dulce (Table 2). Lipid content was lower in forage plants than non-forage plants at both sites (all t-tests, $P = 0.001$) (Table 2). Lipid content of forage plants at Agua Dulce and Charlie Bell were only 27–36% of the lipid contents measured for non-forage plants (Table 2). Values for NDF and

[13] Fox, L.M. 1997. Nutritional content of forage in Sonoran pronghorn habitat, Arizona. M.S. Thesis, The University of Arizona, Tucson, Arizona, USA.

Table 1. Mean percent water content and standard errors (SE) of forage and non-forage plant species sampled at 2 study sites during 5 seasons on Cabeza Prieta National Wildlife Refuge, Arizona from 1995 to 1996. Forage plants were *Ambrosia dumosa*, *Beloperone californica*, *Encelia farinosa*, *Euphorbia* spp., *Hyptis emoryi*, *Janusia gracilis*, *Krameria* spp., *Olneya tesota*, *Opuntia fulgida*, *Prosopis juliflora*, and *Sphaeralcea* spp. Non-forage plants were *Ambrosia deltoidea*, *Jatropha cuneata*, *Larrea tridentata*, and *Sapium biloculare*. Means within rows sharing common uppercase letters are not significantly different for each site at $P < 0.05$. Means with the same lowercase letters are not significantly different within columns at $P < 0.005$ (group alpha level for 10 Bonferroni pairwise comparisons) using 2-way ANOVA on arcsine-transformed data.

	Agua Dulce				Charlie Bell			
	Forage		Non-forage		Forage		Non-forage	
Season	Mean %	SE	Mean %	SE	Mean %	SE	Mean %	SE
Feb-Mar	53.99abc	2.4	45.61	1.5	39.41abcd	1.7	32.77	1.6
Apr-May	46.10ab	2.8	29.41	1.9	47.21bcd	1.6	37.00	1.5
Jun-Jul	49.55ac	3.1	38.17	3.2	35.35acd	2.5	34.52	3.3
Aug-Sep	48.66abc	2.7	55.52	3.9	44.06abc	3.0	44.16	3.4
Oct-Nov	58.36abc	2.5	57.32	2.3	32.63 abd	2.1	34.60	5.8
All	51.59A	1.2	45.49B	1.9	39.73 C	1.1	36.86C	1.8

ADF were from 22 to 29% higher ($P < 0.001$) in forage species than non-forage species at both sites (Table 2). Ash content was greater in forage than non-forage species at Agua Dulce ($P = 0.001$), while no difference occurred in ash content for forage and non-forage species at Charlie Bell. No differences existed in crude protein or NFE content between forage and non-forage species at either site.

Water and Nutrient Intake

Of the 5 diets used to estimate water intake for Sonoran pronghorn, we report only the results of the "no selection" diet, and 1 "selection" diet (Hughes[10] 1991) because these produced the minimum and maximum water intake estimates. Total metabolic water production for each diet ranged from 0.10 to 0.50 liters/animal/day and was ≤20% of the total water intake estimate.

At Agua Dulce, water intake estimates calculated from diet models ranged from 1.85 to 3.39 liters/day for the no selection diet and from 5.42 to 6.89 liters/day for the selection diet (1.5 kg dry matter/animal/day). When the dry matter intake rate was reduced to 1.3 kg/animal/day, water intake estimates ranged from 1.61 to 2.94 liters/day for no selection and from 4.69 to 5.97 liters/day for selection diets (Table 3).

Estimates of water intake for no selection and selection diets at Charlie Bell were similar to or lower than at Agua Dulce for both dry matter intake rates examined. Water intake for no selection and selection diets were similar, and ranged from 1.30 to 2.00 liters/day. These estimates decreased by 13–14% when dry matter intake was reduced from 1.5 to 1.3 kg/animal/day. When only the protein component of metabolic water production was included, water intake estimates decreased by 4–20% (Table 3, column B).

Table 2. Mean percent nutrient content and standard errors (SE) for forage and non-forage vegetation species sampled in 5 periods at 2 study sites from June 1995 to May 1996 on Cabeza Prieta National Wildlife Refuge, Arizona. Forage species were *Ambrosia dumosa, Beloperone californica, Encelia farinosa, Euphorbia* spp., *Hyptis emoryi, Janusia gracilis, Krameria* spp., *Olneya tesota, Opuntia fulgida, Prosopis juliflora*, and *Sphaeralcea* spp. Non-forage species were *Ambrosia deltoidea, Jatropha cuneata, Larrea tridentata*, and *Sapium biloculare*. Means within columns and sites with the same uppercase letters are not significantly different (t-tests, forage vs. non-forage) at $P < 0.05$. Means within columns across sites with the same lowercase letters are not significantly different (t-test, Agua Dulce vs. Charlie Bell) at $P < 0.05$.

Site	Crude protein		N free extract		Lipid		Crude fiber NDF		Crude fiber ADF		Ash	
Forage type	x̄ %	SE	x̄ %	SE	x̄ %	SE	x̄ %	SE	x̄ %	SE	x̄ %	SE
Agua Dulce												
Forage	8.2Aa	0.6	30.3Aa	1.1	3.8Aa	0.4	49.0Aa	1.6	36.1Aa	1.7	8.7Aa	0.6
Non-forage	8.9A	0.6	36.2A	3.0	14.0B	1.9	34.7B	2.0	25.5B	1.9	6.3B	0.3
Charlie Bell												
Forage	8.8Ba	0.6	26.1Bb	1.0	4.3Ca	0.6	53.7Cb	1.6	42.6Cb	1.9	7.1Cb	0.4
Non-forage	8.4B	0.6	31.3B	2.4	12.1D	1.8	42.1D	2.7	32.3D	2.2	6.1C	0.3

Table 3. Water intake (liters/animal/day) estimated for pronghorn from 2 diets, 2 forage intake levels (1.5 and 1.3 kg dry matter/animal/day), 2 study sites, and 5 seasons on Cabeza Prieta National Wildlife Refuge, Arizona from February 1995 to November 1996. A = water intake estimated with metabolic water from protein, carbohydrates, and lipids. B = water intake estimated with metabolic water from protein only.

	Agua Dulce				Charlie Bell			
	No forage[a] Selection		Forage[b] Selection		No forage[a] Selection		Forage[b] Selection	
1.5 kg dry matter/animal/day (water required = 3.4 liters/animal/day)								
Season	A	B	A	B	A	B	A	B
Feb–Mar	2.47	2.10	6.89	6.53	1.49	1.21	1.30	1.06
Apr–May	2.00	1.60	6.08	5.75	1.97	1.64	1.90	1.62
Jun–Jul	1.94	1.57	5.42	5.07	1.54	1.22	1.61	1.30
Aug–Sep	1.85	1.59	6.43	6.17	2.00	1.71	1.65	1.41
Oct–Nov	3.39	3.08	6.14	5.83	1.36	1.11	1.21	0.97
1.3 kg dry matter/animal/day (water required = 1.8 liters/animal/day)								
Feb–Mar	2.14	1.82	5.97	5.66	1.29	1.05	1.12	0.92
Apr–May	1.73	1.39	5.27	4.99	1.71	1.42	1.65	1.40
Jun–Jul	1.68	1.36	4.69	4.39	1.34	1.06	1.39	1.13
Aug–Sep	1.61	1.38	5.58	5.35	1.73	1.49	1.43	1.22
Oct–Nov	2.94	2.67	5.33	5.05	1.17	0.96	1.05	0.84

[a] No selection diets (i.e. forage used in proportion to availability).

[b] Diet based on Hughes (1991).

Based on selective use of forage plants (selection diet), pronghorn met their water requirements (1.8 and 3.4 liters/animal/day) (Richmond et al. 1962) at the Agua Dulce study site in all 5 seasons for both dry matter intake rates. In contrast, random foraging only allowed pronghorn to meet their water requirements at Agua Dulce during February–March and October–November (Table 3). Pronghorn were unable to meet their water requirements at the Charlie Bell site for any diet (selection and no selection), dry matter intake rate, or season.

We reduced the values reported above by 50% to account for water losses due to excretion, respiration, and evaporation. With this reduction, pronghorn consuming 1.5 kg of forage daily were able to meet their water requirements during February–March at Agua Dulce. Smaller pronghorn, consuming 1.3 kg of forage daily, were able to meet their water requirements at the Agua Dulce site during all 5 seasons. When the selection diet models using only protein as the metabolic water component were decreased by half, smaller animals consuming 1.3 kg of forage daily were able to meet their water requirements at the Agua Dulce site during all 5 seasons.

Selection diet model estimates for percent crude protein consumed by pronghorn at the Agua Dulce site ranged from 5.5 to 7.6%. Estimated carbohydrate intake ranged from 32.5 to 36.7% and lipid intake from 2.7 to 3.5% (Table 4). At Charlie Bell, selection diet model estimates for crude protein were slightly higher (i.e., 9.0–11.0%),

Table 4. Nutrient intake (% dietary dry matter) estimated for 5 seasons from 2 diet models. Vegetation sampled on Cabeza Prieta National Wildlife Refuge, NWR, Arizona from June 1995 to May 1996.

Model diet			Agua Dulce		
Element	Feb–Mar	Apr–May	Jun–July	Aug–Sep	Oct–Nov
No selection[a]					
Protein	8.63	6.94	8.49	7.96	11.03
Carbohydrate	31.73	34.88	32.93	21.55	29.16
Lipid	6.66	6.25	6.04	4.85	4.43
Selection[b]					
Protein	6.44	5.49	6.81	6.50	7.57
Carbohydrate	36.67	35.46	36.52	33.90	32.51
Lipid	3.23	2.68	3.46	2.83	3.44
			Charlie Bell		
No selection[a]					
Protein	10.12	10.24	10.53	12.56	10.54
Carbohydrate	26.03	30.17	25.07	24.99	24.11
Lipid	4.26	4.79	6.86	4.50	2.88
Selection[b]					
Protein	9.04	10.97	10.18	10.91	9.42
Carbohydrate	24.79	27.82	27.34	24.96	25.07
Lipid	3.67	4.21	5.57	3.66	2.59

[a]Forage used in proportion to availability.
[b]Dietary selection based on Hughes(1991)

similar for lipids (2.6–5.6%) and lower for carbohydrates (24.8–27.8%). Selection diet model estimates did not show increased values for protein, lipid, or carbohydrate when we compared them to diets without forage selection ($P = 0.05$).

DISCUSSION

Limitations of Diet Models

While constructing diets for each site, season, and selection type, we encountered 3 problems. The first was the lack of complete (year-round) diet data for Sonoran pronghorn. Only 2 studies (AGFD[9] 1981, Hughes[10] 1991) quantified diets for Sonoran pronghorn and these did not cover a calendar year. Other studies (Carr[7,8] 1970, 1972) listed plants consumed by Sonoran pronghorn, but lacked quantitative information. Although the browse species diversity in the study area was low (Fox[13] 1997), we were able to collect several plant species each season that were forage for pronghorn, thereby fully using previous diet information. These sites were representative of Sonoran Desert habitat on the eastern third of CPNWR (Brown 1994) and we collected all forage species found on the sites each season. In spite of the limitations, we were confident this sampling effort was adequate to assess water and nutrient content of forage.

The second problem was the lack of forbs for vegetation sampling and collection due to the drought conditions during our study. In the past, adequate rainfall has occurred on the sites we selected to allow forbs to grow during the summer monsoons (T.L. Cutler, The University of Arizona, personal communication); however, not enough forbs grew on these sites from June 1995 to May 1996 to make collection practical. American pronghorn throughout the western United States make use of forbs as they become seasonally available (Beale and Smith 1970), and forbs are a preferred food in all seasons (Yoakum 1990). As few as 5 species of forbs may constitute from 20 to 99% of American pronghorn diet (Buechner 1950, Beale and Smith 1970, Mitchell and Smoliak 1971, Stephensen et al. 1985, Hughes[10] 1991), depending on rainfall or other factors that influence annual vegetation. The lack of forbs in our diet models has likely made our maximum water intake estimates conservative.

Finally, data are not available on Sonoran pronghorn water requirements in different physiological states (e.g., lactation, pregnancy). We reduced water intake values by 50% to account for physiological water losses and additional stressors.

Use of Free-standing Water

Our data indicate that pronghorn foraging on the eastern third of CPNWR could not meet their water requirements throughout the year through consumption of forage during this drought. Although it was possible for small pronghorn foraging at the Agua Dulce site to meet their water requirements during all 5 seasons, overall water availability in forage plants was too low to sustain larger pronghorn without consumption of free-standing water.

Pronghorn subspecies inhabiting northern environments drink water freely when it is available; however, Sonoran pronghorn have rarely been seen using the limited free-standing water available on CPNWR (Monson 1968, Yoakum 1994). Hughes[10] (1991) suggested that Sonoran pronghorn may occupy habitats where moist forage can be obtained, but further research is necessary to evaluate the perceived need for free-standing water by ungulates when water is scarce (Broyles 1995, Cutler[14] 1996).

The low level of diet variability in preformed water in forage species documented was consistent with previous research on Sonoran Desert vegetation in southern and western Arizona (Alderman et al. 1989). Our data did not support the hypothesis that pronghorn may increase their consumption of preformed water by feeding at a specific time of the day.

Preformed water content for vegetation was, however, affected by site, season (Table 1), and species (Fox[13] 1997). These data were consistent with previous research in Arizona (Hughes and Smith[11] 1990, Hughes[10] 1991), with research in other western states (Mitchell and Smoliak 1971, Yoakum 1994), and with the climatic characteristics of the region (Sellers and Hill 1974, Brown 1994). Water content for forages varied from approximately 5 to 50% among seasons and from 5 to 40% between sites. Seasonally, individual plant species varied widely in preformed water content, presumably reflecting local rainfall and plant phenology, which may influence pronghorn distribution. Other desert ungulates such as mule deer, *Odocoileus heminous eremicus*, in western Arizona moved 30 km across their range to take advantage of rainfall (Rautenstrauch and Krausman 1989). Sonoran pronghorn also move in relation to rainfall and plant succulence (J.J. Hervert, Arizona Game and Fish Department, personal communication).

Previous research supports forage selection by pronghorn on the basis of water content (Beale and Smith 1970, Deblinger and Alldredge 1991, Hughes[10] 1991). We documented higher moisture content in forage species compared to non-forage species at 1 of 2 study sites. Selective foraging for high water content may be important to survival of Sonoran pronghorn in the arid southwest (Hughes and Smith[11] 1990, Hughes[10] 1991, Thompson-Olais[3] 1994, Yoakum 1994), especially if they obtain most of their moisture from plants. However, at the Charlie Bell site, consumption of the available forage did not provide enough moisture for pronghorn. During our investigation, forages that were sampled at the Agua Dulce site could not provide enough moisture to sustain pronghorn in any season. When we assumed a 50% water loss due to physiological functions, the 1.5 kg/animal/day diet provided enough water for large pronghorn only in February–March; the diet for small pronghorn (1.3 kg/animal/day) provided sufficient water in all 5 seasons. The birthing/lactation period for Sonoran pronghorn would, however, create greater water demands. In domestic sheep, water intake by pregnant ewes during early lactation exceeds that of non-pregnant ewes and the water content of milk is higher in lactating

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[14] Cutler, P.L. 1996. Wildlife use of two artificial water developments on the Cabeza Prieta National Wildlife Refuge, southwestern Arizona. M.S. Thesis, The University of Arizona, Tucson, Arizona, USA.

ewes than in non-lactating ewes because of a higher metabolic rate and excretion. Consequently, lactating ewes require twice as much water as dry ewes.

Metabolic water is critical in the water balance of many small desert rodents and may constitute 100% of their water intake (Houpt 1970). Even if 100% of the metabolic water in plants were available, it would account for <20% of the total water produced from forage consumption (preformed plus metabolic). This amount would not be adequate to meet pronghorn requirements.

Our diet models indicated crude protein in simulated pronghorn diets ranged from 5.7 to 10.1% at our 2 sites and may be slightly low when compared to published values for minimum required protein for pronghorn (7–21%) (Schwartz et al. 1977). Crude protein requirements increase beyond maintenance levels during growth, gestation, and lactation (French et al.[15] 1955, NRC 1985). Rumen function may be hindered if crude protein in the diet falls below 7% (Deitz 1965). Sonoran pronghorn foraging on the eastern portion of CPNWR during our study may not have had access to adequate levels of protein.

Estimates of water intake for diet combinations constructed during this study were highly variable by site and season, but in most cases (14 of 20) did not exceed the predicted water requirements. Prior to the reduction of water intake estimates by 50%, Sonoran pronghorn of all sizes could meet their water requirements in all seasons by foraging at the Agua Dulce site. These data indicated that the range inhabited by Sonoran pronghorn may be marginal at times and changes in climatic factors, or the physiological requirements of pronghorn, may be enough to make certain areas unsuitable for pronghorn. Although Sonoran pronghorn have been documented on both sites sampled, they may need to access areas that contain forage higher in moisture, or free-standing water, to survive.

Future research should focus on collecting more comprehensive data about Sonoran pronghorn seasonal habitat use, attributes of forage species, and specific behavioral adaptations. Vegetation associations used by pronghorn should be identified and mapped on a scale compatible with seasonal home-range size, and detailed vegetation sampling stratified by vegetation association should be conducted. The seasonal distribution of pronghorn should be carefully examined in conjunction with habitat information to define areas that can provide adequate forage quality and seasonal water for pronghorn. Without this information, managers cannot make informed decisions about the necessity and placement of water developments or forage improvements. It is also important that research be conducted to determine water requirements of pronghorn across various stages of their life cycle (e.g., pregnancy and lactation) and ambient climatological conditions.

Cutler[14] (1996) did not detect use by pronghorn of 2 water developments on CPNWR that were constructed specifically for that species and noted that those developments may not benefit pronghorn. For water developments to be effective,

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[15] French, C.E., L.C. McEwen, and N.D. Magruder. 1955. Nutritional requirements of white-tailed deer for growth and antler development. U.S. Department of Agriculture, Pacific Agricultural Experiment Station Bulletin 600, Forest Grove, Oregon, USA.

they must be placed in areas accessible to pronghorn when seasonal distributions and physiological needs dictate supplementary use of free-standing water.

If Sonoran pronghorn are to benefit from water developments, water should be added only where it is a limiting factor for the population. Inappropriate addition of water could be detrimental to pronghorn by allowing more water dependent species (e.g., mule deer, burros) to exist where they previously were absent (Bellantoni et al. 1993, Cutler[13] 1996). These other species may compete with pronghorn for forage, provide vectors for disease, prey on pronghorn, or by their presence, deny pronghorn access to water sources (Cutler[14] 1996).

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LITERATURE CITED

Alderman, J.A., P.R. Krausman, and B.D. Leopold. 1989. Diel activity of female desert bighorn sheep in western Arizona. Journal of Wildlife Management 53:264-271.

AOAC. 1990. Official methods of analysis, volume 1, fifteenth edition. Association of the Official Analytical Chemists International, Arlington, Virginia, USA.

Beale, D.M. and A.D. Smith. 1970. Forage use, water consumption, and productivity of pronghorn antelope in western Utah. Journal of Wildlife Management 34:570-582.

Bellantoni, E.S., P.R. Krausman, and W.W. Shaw. 1993. Desert mule deer use of an urban environment. Transactions of the North American Wildlife and Natural Resource Conference 58:92-101.

Brown, D.E. 1994. Biotic communities: Southwestern United States and northwestern Mexico. University of Utah Press, Salt Lake City, Utah, USA.

Broyles, B. 1995. Desert wildlife water developments: Questioning use in the Southwest. Wildlife Society Bulletin 23:663-675.

Buechner, H.K. 1950. Life history, ecology, and range use of the pronghorn antelope in Trans-Pecos Texas. American Midland Naturalist 43:257-354.

Cook, J.G. and five others. 1996. Nutrition-growth relations of elk calves during late summer and fall. Journal of Wildlife Management 60:528-541.

Daubenmire, R. 1959. A canopy coverage method of vegetation analysis. Northwest Science 33:43-64.

Deblinger, R.D. and A.W. Alldredge. 1991. Influence of free water on pronghorn distribution in the sagebrush/steppe grassland. Wildlife Society Bulletin 19:321-326.

Deitz, D.R. 1965. Deer nutrition research in range management. Transactions of the North American Wildlife and Natural Resource Conference 30:274-285.

Einarsen, A.S. 1948. The pronghorn antelope and its management. Wildlife Management Institute, Washington, D.C., USA.

Friedel, M.H., V.H. Chewings, and G.N. Bastin. 1988. The use of comparative yield and dry-weight-rank techniques in arid rangelands. Journal of Range Management 41:430-434.

Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analyses. U.S. Department of Agriculture Handbook 379, Washington, D.C., USA.

Goldman, E.A. 1945. A new pronghorn antelope from Sonora. Proceedings of the Biological Society of Washington 50:3-4.

Goodson, N.J., D.R. Stevens, and J.A. Bailey. 1991. Effects of snow on foraging ecology and nutrition of bighorn sheep. Journal of Wildlife Management 55:214-222.

Haufler, J.B. and F.A. Servello. 1994. Techniques for wildlife nutritional analyses. Pages 307-323 *in*: T.A. Bookhout, editor. Research and management techniques for wildlife and habitats, fifth edition. The Wildlife Society, Bethesda, Maryland, USA.

Haydock, K.P. and N.H. Shaw. 1975. The comparative yield method for estimating dry matter yield of pasture. Australian Journal of Experimental Agriculture and Animal Husbandry 15:663-670.

Higgins, K.F., J.L. Oldemeyer, K.J. Jenkins, G.K. Clambey, and R.F. Harlow. 1994. Vegetation sampling and measurement. Pages 567-591 *in*: T.A. Bookhout, editor. Research and management techniques for wildlife and habitats, fifth edition. The Wildlife Society, Bethesda, Maryland, USA.

Hobbs, N.T. and D.M. Swift. 1985. Estimates of habitat carrying capacity incorporating explicit nutritional constraints. Journal of Wildlife Management 49:814-822.

Houpt, T.R. 1970. Water, electrolytes, and acid-base balance. Pages 743-766 *in*: M.J. Swenson, editor. Duke's physiology of domestic animals, eighth edition. Cornell University Press, Ithaca, New York, USA.

Knox, K.L., J.G. Nagy, and R.D. Brown. 1969. Water turnover in mule deer. Journal of Wildlife Management 33:389-393.

Lehr, H.J. 1978. A catalogue of the flora of Arizona. Northland Press, Flagstaff, Arizona, USA.

Leopold, A. 1933. Game management. Charles Scribner's Sons, New York, New York, USA.

Mazaika, R. and P.R. Krausman. 1991. Use of dry-weight-rank multipliers for desert vegetation. Journal of Range Management 44:409-411.

Mazaika, R., P.R. Krausman, and R.C. Etchberger. 1992. Forage availability for mountain sheep in Pusch Ridge Wilderness, Arizona. Southwestern Naturalist 37:372-378.

Mitchell, G.J. and S. Smoliak. 1971. Pronghorn antelope range characteristics and food habits in Alberta. Journal of Wildlife Management 35:238-250.

Monson, G. 1968. The desert pronghorn. Desert Bighorn Council Transactions 12:63-69.

NRC. 1985. Nutrient requirements of domestic animals: Nutrient requirements of sheep, sixth edition. National Research Council, Subcommittee on Sheep Nutrition. National Academy Press, Washington, D.C., USA.

O'Gara, B.W. and J.D. Yoakum. 1990. Additional capture methods and habitat suitability criteria for pronghorn translocations. Pronghorn Antelope Workshop Proceedings 14:51-62.

O'Gara, B.W. and J.D. Yoakum, editors. 1992. Pronghorn management guidelines. Pronghorn Antelope Workshop Proceedings 15:1-110.

Rautenstrauch, K.R. and P.R. Krausman. 1989. Influence of water availability and rainfall on movements of desert mule deer. Journal of Mammalogy 70:197-201.

Richmond, C.R., W.H. Langham, and T.T. Trujillo. 1962. Comparative metabolism of tritiated water by mammals. Journal of Cellular and Comparative Physiology 59:45-53.

Robbins, C.T. 1993. Wildlife feeding and nutrition, second edition. Academic Press Inc., San Diego, California, USA.

Robertson, J.B. and P.J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Page 123 *in*: W.P. James and O. Theander, editors. The analysis of dietary fiber in food. Marcel Dekker, New York, New York, USA.

Roebuck, C.M., C.D. Simpson, and B.E. Dahl. 1982. Food habits and preferences of pronghorn in three habitats of the Texas Panhandle. Pronghorn Antelope Workshop Proceedings 10:71-78.

Roundy, B.A. and G.B. Ruyle. 1989. Effects of herbivory on twig dynamics of a Sonoran Desert shrub *Simmondsia chinensis* (Link) Schn. Journal of Applied Ecology 26:701-710.

Ruyle, G.B., editor. 1990. Some methods for monitoring rangelands and other natural area vegetation. College of Agriculture, Extension Report No. 9043. The University of Arizona, Tucson, Arizona, USA.

Schwartz, C.C. and J.G. Nagy. 1976. Pronghorn diets relative to forage availability in northeastern Colorado. Journal of Wildlife Management 40:469-478.

Schwartz, C.C. and R.W. Rice. 1977. Pronghorn dietary quality relative to forage availability and other ruminants in Colorado. Journal of Wildlife Management 41:161-168.

Sellers, W.D. and R.H. Hill. 1974. Arizona climate-1931–1972. The University of Arizona Press, Tucson, Arizona, USA.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry, third edition. W.H. Freeman and Company, New York, New York, USA.

Stephenson, T.E., J.L. Holechcek, and C.B. Kuykendall. 1985. Drought effect on pronghorn and other ungulate diets. Journal of Wildlife Management 49:146-151.

't Mannetje, L. and K.P. Haydock. 1963. The dry-weight-rank method for the botanical analysis of pasture. Journal of the British Grassland Society 18:268-275.

Wallmo, O.C. and W.L. Regelin. 1981. Rocky mountain and intermountain habitats: Food habits and nutrition. Pages 387-398 *in*: O.C. Wallmo, editor. Mule and black-tailed deer of North America. University of Nebraska Press, Lincoln, Nebraska, USA.

Yoakum, J. 1978. Pronghorn. Pages 103-122 *in*: J.L. Schmidt and D.L. Gilbert, editors. Big game of North America: Ecology and management. Stackpole Books, Harrisburg, Pennsylvania, USA.

Yoakum, J. 1986. Trends in pronghorn populations: 1800–1983. Pronghorn Antelope Workshop Proceedings 12:77-85.

Yoakum, J. 1990. Food habits of the pronghorn. Pronghorn Antelope Workshop Proceedings 14:102-111.

Yoakum, J. 1994. Water requirements for pronghorn. Pronghorn Antelope Workshop Proceedings 16:143-157.

Yoakum, J., B.W. O'Gara, and V.W. Howard Jr. 1996. Pronghorn on western rangelands. Pages 211-226 *in*: P.R. Krausman, editor. Rangeland wildlife. Society of Range Management, Denver, Colorado, USA.

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Appendix 1. Formulas used to calculate percent composition, biomass, and total metabolic water produced by forage species consumed by pronghorn.

Relative % composition of each plant species in diet:

(% actual composition species A x 100) ÷ % total composition all species = relative % species A

Dry matter (DM) for each species in diet:

Relative % species A x 1.5 kg (or 1.3 kg) = DM (kg) species A

Original (wet) mass for each species in diet:

DM (g) Species A x (% preformed water in species A ÷100) ÷ (100% preformed water species A÷ 100) = wet weight (g) of species A

Metabolic water production for each forage species in diet:

DM(g) x̄ % crude protein/100 x 0.4[a] + DM(g) x̄ % carbohydrate/100 x 0.56[a] + DM(g) x̄ % lipid/100 x 1.07 = Total metabolic water from species A (g)

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[a] Values from Robbins (1993).

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PREVALENCE OF *MYCOBACTERIUM* IN WILD AND CAPTIVE DELTA SMELT

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Infections associated with *Mycobacterium* were examined in wild and captive delta smelt, *Hypomesus transpacificus*. *Mycobacterium* was not detected from any of the fish examined immediately after collection from the Sacramento-San Joaquin Estuary or during captivity of broodstock groups at water temperatures of 9–12°C. However, *Mycobacterium* was isolated from, and mycobacteriosis occurred in, broodstock held at 16°C during spawning season and in experimental groups maintained at 17°C. *Mycobacterium* and mycobacteriosis were more prevalent among groups frequently handled for physiological experiments. Broodstock groups that were less stressed exhibited a lower prevalence of the bacterium and the disease. These findings suggest that *Mycobacterium* may be present in a latent state in the wild population of delta smelt and infections may progress from asymptomatic to clinical under intensive culture conditions. Two species of non-pigmented and rapidly growing *Mycobacterium* (Runyon Group IV) were isolated from the liver, spleen, and kidney tissues. The most abundant isolates were identified as *Mycobacterium chelonae* and a less dominant group was designated as *Mycobacterium* sp., based on biochemical and growth properties and chromatography of lipids from representative strains. A disease control strategy that combined rearing of small isolated groups at lowered water temperatures, minimal handling, and periodic prophylactic treatments after handling minimized the impact of *Mycobacterium* in delta smelt broodstock reared in captivity.

INTRODUCTION

The abundance of delta smelt, *Hypomesus transpacificus*, an osmerid fish endemic to the Sacramento-San Joaquin Estuary, California, has declined (Sweetnam 1999). Habitat destruction or degradation associated with inadequate freshwater inflow, availability of food supply, invasion of exotic species, and other environmental factors have contributed to this decline (Moyle et al. 1992). These factors are similar to those that have affected striped bass in the estuary – water export, decreased freshwater

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inflow, decreased spawning stocks, and changes in food supply due to invasion of exotic species (Stevens et al. 1985).

Most vertebrates, amphibians, and reptiles (Fryer and Rohovec 1984, Good 1985), and probably all fish species (Belas et al. 1995, Noga 1995, Bruno et al. 1998), are susceptible to *Mycobacterium*. Infections due to *Mycobacterium* have been described from over 150 species of marine, brackish, and freshwater fishes throughout the world (Nigrelli and Vogel 1963) including striped bass, *Morone saxatilis,* in the Sacramento-San Joaquin Estuary (Sakanari et al. 1983, Hedrick et al. 1987). Several species of *Mycobacterium* found in fish are now known as human pathogens (Popp and Reichenbach-Klinke 1982, Collins et al. 1985).

In a preliminary study, delta smelt broodstock suffering from severe infections due to *Mycobacterium* did not successfully spawn. Most of the infected fish subsequently died and survivors were sacrificed to control the spread of the bacterium (Mager[5] 1996). The goals of the current study were to determine the prevalence of *Mycobacterium* and mycobacteriosis in delta smelt upon collection from the Sacramento-San Joaquin Estuary and to evaluate the progress of infections after holding fish in captivity for broodstock development and physiological experiments.

METHODS

Fish examined for *Mycobacterium* infections were from 2 separate studies, 1 for broodstock development and 1 for physiological experiments. The specific design and findings from these broodstock and physiological studies have been previously reported (Mager[5] 1996, Swanson et al.[6], in review).

Delta smelt were collected from moderate depth to shallow open waters (<6 m deep) of the western delta near the confluence of the Sacramento and San Joaquin rivers. Fish were collected with a 3-mm-mesh seine during late summer and fall using methods described in Swanson et al. (1996). Following collection, fish were transported in oxygenated cylindrical polyethylene bags to the Aquatic Center laboratory at the University of California, Davis, California. Fish remained in the transport bags at 9°C and were kept in tanks at 1–3°C below this temperature for 2–8 days following collection. Water salinity was maintained between 2 and 8‰ by adding a concentrated sodium chloride solution. Fish were shifted to well water following initial acclimation. To prevent the spread of infection associated with *Mycobacterium* and other potential fish pathogens, groups collected during different sampling periods were held in separate rearing tanks. In addition, all fish were given prophylactic treatments with Nitrofurazone (2–10 mg/liter) and formalin (0.1 ml/liter) baths administered for 1 hour on alternate days for 4 days. Groups of delta

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[5] Mager, R.C. 1996. Gametogenesis, reproduction and artificial propagation of delta smelt, *Hypomesus transpacificus*. Ph.D. Dissertation, University of California, Davis, California, USA.

[6] Swanson, C., D.B. Antonio, P.S. Young, J.J. Cech Jr., and R.P. Hedrick. In review. Reduced swimming performance in delta smelt infected with *Mycobacterium* spp. Transactions of the American Fisheries Society.

smelt were then held in 2 different and entirely independent culture systems designed for broodstock development and physiological experiments. Both broodstock and experimental fish were fed brine shrimp, *Artemia* sp., and a commercial diet with automatic feeders. Waste food and feces were removed daily by siphoning.

Delta smelt (initial mean weight 0.65 g) held for rearing to the broodstock stage (weight 2–5 g) were kept at the Aquatic Center in four 265-liter, circular, fiberglass tanks. The final stocking density was 2.3 g/liter. Each tank was separated by plastic sheets suspended from the ceiling as a precaution for possible airborne transmission of *Mycobacterium* and had designated separate sets of tools for cleaning and netting that were routinely disinfected in 100-ppm iodine solution. The tanks were supplied with recirculating water (0.5–1.0 liter/minute) that was drained into a 130-liter sump, pumped through a 25-μm particle sand biofilter and 373-watt chiller (Aquanetics Inc.[7], San Diego, California, USA) and treated with ultraviolet light. Water temperature was lowered incrementally (weekly) from 16°C in November to 9°C through January and then increased to 16°C during spawning from April to June. After spawning, water temperature was maintained at <17°C. As these groups were held for spawning, the fish were not moved nor disturbed except during daily tank cleaning and sampling. Live fish were sampled periodically from each group for dissection and monitoring of gonadal development and growth (Mager[5] 1996). Moribund or dead fish were collected immediately and submitted to the Fish Health Laboratory (FHL), University of California, Davis, for necropsy.

Groups of 6–8-month-old delta smelt (2–5 g) were used for measurements of different physiological variables, including swimming performance. The fish were held at the Aquatic Center in 200-liter circular tanks supplied with non-chlorinated flow-through well water. Water temperature at 9°C was increased 1°C daily and then maintained at 12 or 17°C. As fish from these groups were used for experimental purposes, they were repeatedly subjected to handling stress and occasionally moved among tanks. However, the original number of animals was recorded in each tank for evaluating infection prevalence at the end of the study. The approximate stocking density in each tank was 1.5 g/liter. Group 1 (n = 60) was held continuously at 17°C for various physiological studies. Groups 2, 3, and 4 were held for swimming performance measurements. Group 2 (n = 60) was continuously held at 17°C whereas Group 3 (n = 50) was maintained at 12°C. Group 4 (n = 50) was initially held at 17°C for 1 month and then shifted to 12°C. Sampled fish were transported live in aerated buckets or, if dead, on ice to the FHL for necropsy. Moribund fish or fish that succumbed to infections during the study were also included for necropsy.

Delta smelt were anesthetized with 50 mg/liter ethyl p-amino-benzoate (Benzocaine, Sigma[7], St. Louis, Missouri, USA) and then examined for the presence of parasites on the gills and body surface by microscopic examination of smears prepared from these tissues. The ventral portion was then swabbed with 70% ethyl alcohol and incisions made in the abdomen with a sterile scalpel. Using a sterile loop, spleen, liver, and kidney tissues were directly inoculated onto

[7] Use of trade names does not imply endorsement by the California Department of Fish and Game.

Lowenstein-Jensen medium (PML Microbiologicals[7], Tualatin, Oregon, USA) for initial isolation of *Mycobacterium* and on blood agar plates for other bacteria. Cultures were incubated at 25°C and examined for growth of rapidly growing *Mycobacterium* at 5 days postinoculation and then weekly until 6 months for slow-growing isolates. Tissue imprints were also prepared on glass slides, air-dried, heat-fixed, and stained by the acid fast Ziehl-Neelsen (ZN) method (Difco[7], Detroit, Michigan, USA). The presence of *Mycobacterium* was periodically examined in live, dead, or moribund fish, and survivors at the end of the studies as described above.

Isolates originally grown on Lowenstein-Jensen medium were subcultured onto Middlebrook 7H10 agar (Difco[7]) and then examined for their growth and biochemical properties (Wayne and Kubica 1986). Chromogenicity of colonies on Lowenstein-Jensen agar was tested at 37°C. Representative isolates were sent to a level III *Mycobacterium* laboratory at the National Veterinary Services Laboratory at Ames, Iowa to confirm identification by lipid chromatography.

Susceptibility to anti-mycobacterial drugs was conducted using the Kirby-Bauer method (Bauer et al. 1996). Representative isolates were suspended in sterile saline solution equivalent to a 0.5 McFarland turbidity standard (Hardy Diagnostics[7], Santa Maria, California, USA). MiddleBrook 7H10 agar and Mueller-Hinton (Difco[7]) plates were inoculated with each bacterial suspension (100 µl) and then overlaid with discs that were impregnated with specific concentrations of antibiotics. The size of the inhibitory zone around the disc following incubation at 25°C for 1 month measured the susceptibility of the bacterium to the drug (Bauer et al. 1996). Anti-mycobacterial drugs examined included Rifampin (5 µg), Imipenem (10 µg), Isoniazid (1 µg) and Clarithromycin (15 µg) (Remel[7], Lenexa, Kansas, USA).

Tissues or whole fish were fixed in Davidson's solution for 24 hours, changed to 70% ethyl alcohol, and then processed for routine histology (Humason 1979). Microscopic changes in organ tissues of delta smelt infected with *Mycobacterium* were evaluated by light microscopy of histological sections stained with hematoxylin and eosin and ZN acid-fast reagents.

RESULTS

Random samples of delta smelt examined after collection from the estuary and prior to holding in the broodstock and experimental systems did not reveal the presence of *Mycobacterium* or other bacterial pathogens and parasites. Fish that died during the early rearing period were culture negative for *Mycobacterium* and the cause of death was attributed to transportation stress and inappetence.

Broodstock fish first succumbed to infection with *Mycobacterium* after 6–7 months in captivity when water temperatures were increased to 16°C during the spawning season (April–June). The prevalence of *Mycobacterium* in broodstock (n = 120 fish/group) was 0.0, 1.6, 21.6, and 18.3% for Groups 1, 2, 3 and 4, respectively (Table 1).

Mortalities due to *Mycobacterium* infections occurred in the experimental groups following at least 1 month in captivity at 17°C. At the end of the study, a high prevalence of *Mycobacterium* was found in groups continuously held at 17°C (Table 1), ranging from 36.6% to 50.0% for Group 2 (n = 60) and Group 1 (n = 60). A high

prevalence of the bacterium (42.0%) was also observed even after water temperature was decreased from 17 to 12°C (Group 4, n = 50). *Mycobacterium* was not detected from fish continuously maintained at 12°C (Group 3, n = 50). Moderate to severe clinical signs of disease, including emaciation, exophthalmos, and white nodules in the liver, were more prevalent in the experimental groups than *Mycobacterium*-infected broodstock.

Mycobacterium was easily cultured 7–10 days following inoculation of infected tissues on Lowenstein-Jensen medium. *Aeromonas*, usually present as normal bacterial flora, grew on blood agar plates 2–3 days after inoculation of the tissues. Imprints prepared from the liver, spleen, and kidney of moribund or live fish showed acid-fast rods measuring 0.5 x 2.5 µm. On Lowenstein-Jensen medium, colonies were smooth, shiny, and non-chromogenic in the presence or absence of light at 37°C. All isolates grew optimally between 20 and 30°C compared to 15 or 37°C. Initially classified under Runyon Group IV due to rapid growth (Wayne and Kubica 1986), the isolates were subsequently identified as 2 different species based on growth, biochemical characteristics (Table 2), and lipid chromatography. The dominant isolates were identified as *M. chelonae*; however, characteristics of the less dominant group were not conclusive and hence it was designated as *Mycobacterium* sp. The presence of the pathogen among the groups as indicated in Table 1 represents the combined prevalence of both species, *M. chelonae* and *Mycobacterium* sp. While *M. chelonae* isolates were not inhibited in vitro with any of the drugs tested except Clarithromycin, *Mycobacterium* sp. were susceptible to both Rifampin and Clarithromycin (Table 2).

Table 1. Prevalence of *Mycobacterium* in delta smelt from the Sacramento-San Joaquin Estuary following periods of captivity.

Group		No. fish positive for *Mycobacterium*[a]	No.fish/tank	% prevalence
Broodstock groups				
1	-	0	120	0.0
2	+ 2 dead[b]	2	120	1.6
3	+ 26 live	26	120	21.6
4	+ 22 live	22	120	18.3
Experimental groups				
1	17°C	30	60	50.0
2	17°C	22	60	36.6
3	12°C	0	50	0.0
4	17– 12°C[c]	21	50	42.0

[a] *Mycobacterium* were isolated from the spleen, kidney, and liver by direct inoculation of these tissue samples onto Lowenstein–Jensen medium.

[b] Indicates presence (+) or absence (-) of *Mycobacterium* from live or dead broodstock fish examined. Broodstock groups were held at 9–12°C for 6–8 months and the temperature raised to 16°C during spawning.

[c] Fish were held at 17°C for 1 month and then moved to 12°C.

Microscopic changes observed among delta smelt from which *Mycobacterium* were isolated ranged from mild to severe. Severe hyperplasia of the branchial epithelium and deformed cartilage were evident in the primary gill filaments (Fig. 1A). The snout frequently showed moderate to severe ulcerative lesions characterized by a diffuse granulomatous response in the underlying dermis (Fig. 1B). Several small foci of macrophages were interspersed among the interstitium of the anterior kidney (Fig. 1C). Colonial aggregates of *Mycobacterium* were detected in ZN-stained sections of the cardiac muscle of the ventricle (Fig. 1D) associated with focal necrosis of hepatocytes in the liver (Fig. 1E) and with little host response in the submucosa of the large intestine (Fig. 1F).

DISCUSSION

Our observations suggest that wild populations of delta smelt harbor subclinical infections of *Mycobacterium*. These infections progress to clinical infections presumably due to handling or culture conditions during captivity. Strict isolation of fish groups and disease preventive measures were, however, effective in

Table 2. Biochemical and growth characteristics and anti-microbial sensitivity of *Mycobacterium* isolates from delta smelt.

Test	*M. chelonae*	*Mycobacterium* sp.
Growth in 7 days	+[a]	+
Growth in 5% NaCl	+	+
Pigment production (37°C)	–	
Urease	+	+
Nitrate reduction	–	
Catalase	–	+
Tween-80 hydrolysis	(5, 10 days)[b]	(5, 10 days)
Arylsulfatase	+ (3, 14 days)[c]	+ (3, 14 days)
Pyrazinamidaze	+ (4 days)	+ (4 days)
Niacin		
In vitro anti-microbial sensitivity[d]		
Rifampin 5 µg	resistant	susceptible
Imipenem 10 µg	resistant	resistant
Izoniazid 1 µg	resistant	resistant
Clarithromycin 15 µg	susceptible	susceptible

[a] Bacteria showed a positive response (+) or a negative response (–) to the test following 1 month of incubation at 25°C.

[b] Time required for Tween 80 hydrolysis varies from 5-10 days in different species.

[c] *M. chelonae* complex have rapid arylsulfatase activity that can be detected in 3 days while other species such as *M. marinum*, exhibit the activity in 14 days.

[d] Susceptible organisms showed inhibition zones, suggesting that infection due to the organism maybe treated with the concentration of the antimicrobial used. Resistant organisms did not demonstrate inhibition zones, indicating that the drug is not clinically effective for treatment of the bacterium.

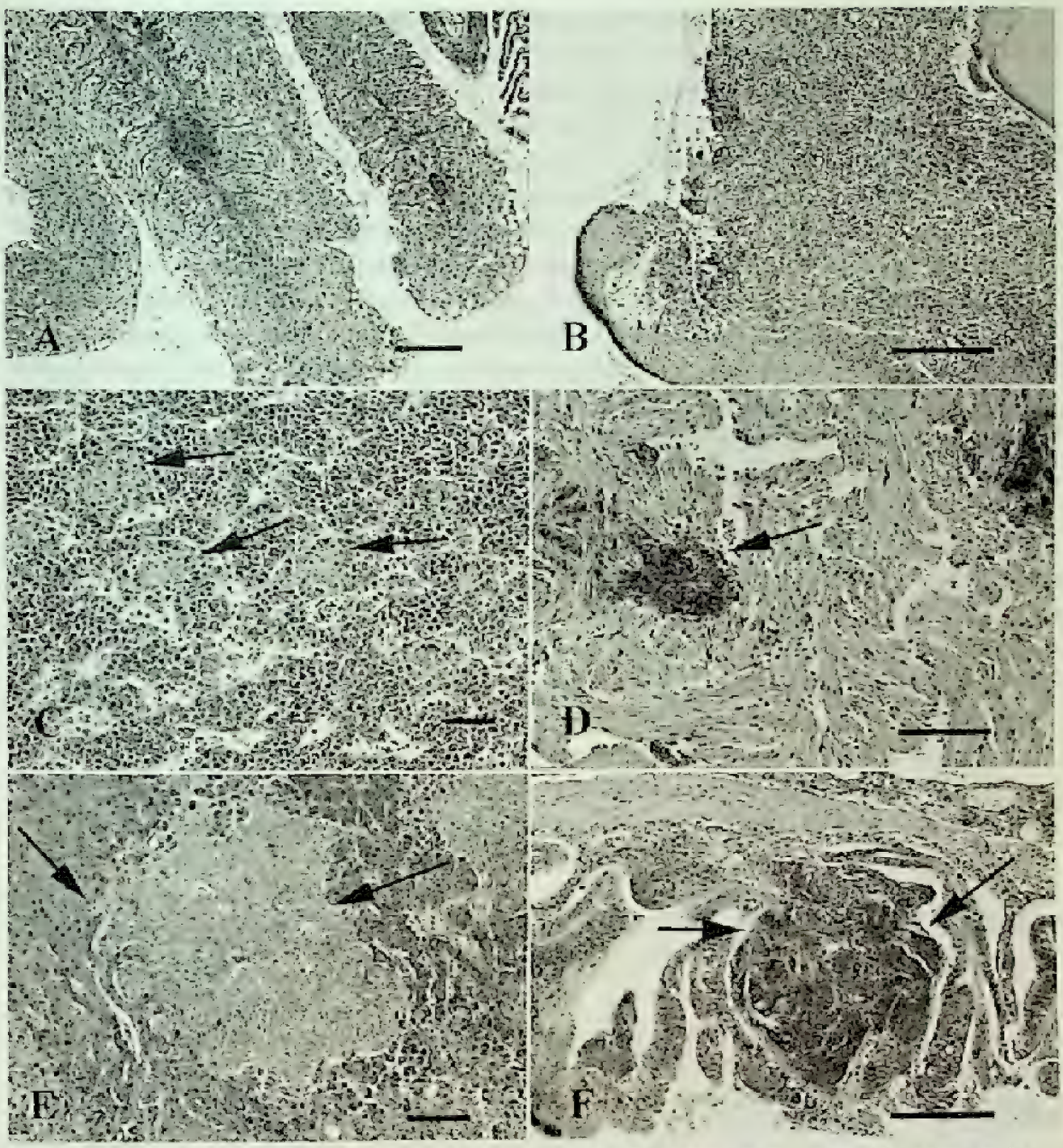

Figure 1. Microscopic lesions in captive delta smelt associated with *Mycobacterium*. (A) Hyperplasia of the branchial epithelium and deformation of supporting cartilage in the primary filament, (B) ulcerative lesion on the snout with underlying granulomatous response in the dermis, (C) several foci (arrows) of macrophage aggregates interspersed among the interstitium of the anterior kidney, (D) colony of *Mycobacterium* spp. (arrows) in the cardiac muscle of the ventricle, (E) focal necrosis in the parenchyma of the liver (arrows), and (F) a bacterial colony in the submucosa of the large intestine. A, B, and C are hematoxylin and eosin- stained sections; D, E, and F are stained withZiehl-Neelsen acid-fast reagents. Bars for A, B, D, E, and F = 100 µm and bar for C = 50 µm.

minimizing the impact of *Mycobacterium* in captive delta smelt held for broodstock development. In contrast to the broodstock groups, experimental groups, including those for physiological experiments that were not subjected to rigorous disease control protocols, eventually developed severe infections. Preventive measures employed

in our studies included isolation and prophylactic bath treatments with nitrofurazone and formalin of newly collected fish groups, rigorous necropsy to identify infected populations, and reduced fish handling and other stress factors. Preventive approaches are critical, as most therapeutic treatments for mycobacteriosis are ineffective (Belas et al. 1995, Noga 1995).

The prevalence and severity of *Mycobacterium* infections in delta smelt appeared to be temperature dependent (Table 1). The highest prevalences of *Mycobacterium* (50.0% and 36.6%) occurred in experimental groups maintained at 17°C. Fish succumbed to specific infections with the bacterium as early as 1 month after holding at this temperature. In the broodstock groups, the bacterium and the disease were observed only after 6–7 months in captivity when water temperatures were increased to 16°C during spawning. Once established, infections persisted (42.0%), even when water temperature was decreased from 17 to 12°C. A constant water temperature of 12°C maintained throughout the study did not promote the onset of *Mycobacterium* infections.

The in vitro growth of both *Mycobacterium* sp. and *M. chelonae* was optimal between 20 and 30°C in our studies. In studies with salmonids, infections associated with *M. chelonae* showed greater mortality at 18°C compared to groups maintained at 12°C (Arakawa and Fryer 1984). The severity and spread of infections with *Mycobacterium* observed in delta smelt are likely exacerbated at temperatures favorable for replication of the pathogen as reported for *M. marinum* (Ramakrishnan and Falkow 1994).

As *Mycobacterium* was not detected from fish immediately after capture, the source of infection is not conclusive. However, infections and disease due to *Mycobacterium* were observed in 2 independent studies (broodstock and physiological experiments) that used cohorts held in independent culture systems. *Mycobacterium* and the disease were not detected in 1 group of broodstock fish despite the identical holding conditions among all broodstock groups. A low prevalence of *Mycobacterium* in Group 2 (1.6%) was observed after 2 dead fish infected with the bacterium were removed in the late phase of rearing; survivors were negative for the bacterium. On the other hand, although no delta smelt succumbed to infection during the experiment, *Mycobacterium* in broodstock Groups 3 and 4 had prevalences of 21.6% and 18.3%, at the termination of the study, indicating that subclinical infections were present.

Although transmission routes are unclear, *Mycobacterium* may have been transmitted from subclinically infected delta smelt to healthy fish in the same group during captivity. Infected fish can directly shed mycobacterial cells from intestines as shown in our study (Fig. 1F), skin ulcers, fecal products, or dead carcasses (Hedrick et al. 1987, Belas et al. 1995, Noga 1995). *Mycobacterium* can also survive for up to 2 years in the environment (Reichenbache-Klinke 1972). Transmission by ingestion is probably the major source of infection (Noga 1995), although vertical transmission from broodfish to their progeny has also been suggested (Nigrelli and Vogel 1963, Ashburner 1977).

Risk factors responsible for infections with *Mycobacterium* in delta smelt are not known. Dissolved oxygen (8-10 mg/liter) and stocking densities (1.5-2.3 g/liter) were kept to satisfactory levels in all tanks. Stress from handling and spawning

and increased water temperatures appear to be the principal contributors to the increased prevalence and severity of infections among fish in certain groups in our study. Intensive culture and handling can exacerbate disease among fish populations (Smith 1996). The stress associated with these factors is known to reduce immune competence in several fish species (Schreck 1996) and may have predisposed the delta smelt in our study to mycobacteriosis.

The majority of the isolated bacteria from delta smelt were identified as *M. chelonae* and the less dominant forms that could not be identified (*Mycobacterium* sp.) were occasionally recovered from moribund or dead fish. Identification of *Mycobacterium* species from fish is difficult (Colorni 1992, Hatai et al. 1993). Infections with *M. chelonae* are well documented in several species of salmonids (Arakawa and Fryer 1984, Humphrey et al. 1987, Bruno et al. 1998) and in ornamental fish (Bernstad 1974, Landsdell et al. 1993, McCormick et al. 1995). Granuloma formation is a dominant pathological characteristic of fish mycobacteriosis (Balouet and Baudin Laurencin 1986, Landsdell et al. 1993, Smith 1996) that was also evident among delta smelt from which mycobacteria were isolated.

The presence of *Mycobacterium* severely impaired the swimming performance of delta smelt (Swanson et al.[6], in review). In contrast, groups of captive broodstock that did not suffer infections with *Mycobacterium* spp. were successfully spawned in captivity (Mager[5] 1996). To control mycobacteriosis, both disinfection procedures and quarantine are critical (Noga 1995). In our studies, multiple isolated rearing systems, proper temperature regimes, and reduced handling stress played key roles in controlling infections with *Mycobacterium* spp. in delta smelt.

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LITERATURE CITED

Arakawa, C.K. and J.L. Fryer. 1984. Isolation and characterization of a new subspecies of *Mycobacterium chelonei* infectious for salmonid fish. Helgolander Wiss Meersunters 37:329-342.

Ashburner, L.D. 1977. Mycobacteriosis in hatchery-confined chinook salmon (*Oncorhynchus tshawytscha*) Walbaum, in Australia. Journal of Fish Biology 10:523-528.

Balouet, G. and F. Baudin Laurencin. 1986. Granulomatous nodules in fish: An experimental assessment in rainbow trout, *Salmo gairdneri* Richardson, and turbot, *Scophthalmus maximus* (L.). Journal of Fish Diseases 9:417-429.

Bauer, A.W., W.M. Kirby, and J.C. Sherris. 1996. Antibiotic susceptibility testing by a standardized single disk method. Journal of Clinical Pathology 45:493-496.

Belas, R., P. Faloon, and A. Hannaford. 1995. Potential applications of molecular biology to the study of fish mycobacteriosis. Annual Review of Fish Diseases 5:133-173.

Bernstad, S. 1974. *Mycobacterium borstelense* isolated from aquarium fishes with

tuberculosis lesions. Scandinavian Journal of Infectious Diseases 6:241-246.

Bruno, D.W. and 6 others. 1998. Pathology attributed to *Mycobacterium chelonae* infection among farmed and laboratory-infected Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms 33:101-109.

Collins, C., J. Grange, and W. Noble. 1985. *Mycobacterium marinum* infections in man. Journal of Hygiene Cambridge 94:135-149.

Colorni, A. 1992. A systemic mycobacteriosis in the European sea bass *Dicentrarchus labrax* cultured in Eilat (Red Sea). Israeli Journal of Aquaculture Bamidgeh 44:75-81.

Fryer, J.L. and J.S. Rohovec. 1984. Principal bacterial diseases of cultured marine fish. Pages 533-545 *in*: O. Kinne and H.P. Bulnhiem, editors. Diseases of marine organisms. Verlag, Helgoland, Germany.

Good, R.C. 1985. Opportunistic pathogens in the genus *Mycobacterium*. Annual Review of Microbiology 39:347-369.

Hatai, K., O. Lawhavinit, K. Toda, and Y. Sugou. 1993. *Mycobacterium* infection in pejerrey, *Odonthetes bonariensis* Cuvier & Valenciennes. Journal of Fish Diseases 16:397-492.

Hedrick, R.P., T. McDowell, and J. Groff. 1987. Mycobacteriosis in cultured striped bass from California. Journal of Wildlife Diseases 23:391-395.

Humason, G.L. 1979. Animal tissue techniques. W.H. Freeman & Co., San Francisco, California, USA.

Humphrey, J.D., C.E. Lancaster, N. Gudkovs, and J.W. Copeland. 1987. The disease status of Australian salmonids: Bacteria and bacterial diseases. Journal of Fish Diseases 10:403-410.

Landsdell, W., B. Dixon, N. Smith, and L. Benjamin. 1993. Isolation of several *Mycobacterium* species from fish. Journal of Aquatic Animal Health 5:73-76.

McCormick, J.I., M.S. Hughes, and M.F. McLoughlin. 1995. Identification of *Mycobacterium chelonae* in a cichlid oscar, *Astronotus ocellatus* Cuvier, by direct cycle sequencing of polymerase chain reaction amplified 16srRNA gene sequences. Journal of Fish Diseases 18:459-461.

Moyle, P.B., B. Herbold, D.E. Stevens, and L.W. Miller. 1992. Life history and status of delta smelt in the Sacramento-San Joaquin Estuary, California. Transactions of the American Fisheries Society 121:67-77.

Nigrelli, R.F. and H. Vogel. 1963. Spontaneous tuberculosis in fishes and in other cold-blooded vertebrates with special reference to *Mycobacterium fortuitum* Cruz from fish and human lesions. Zoologica 48:130-143.

Noga, E.J. 1995. Fish disease: Diagnosis and treatment. Mosby-Year Book, Inc., St. Louis, Missouri, USA.

Popp, W. and H.H. Reichenbach-Klinke. 1982. Fish pathogenic bacteria as source of human infection with special reference to the "Swimming Pool Granuloma." Pages 3-8 *in*: W. Ahne, editor. Aspects of human health in connection with fish and fish food. Contributions to fish biology. Gustav Fischer Verlag, Stuttgart, Germany.

Ramakrishnan, L. and S. Falkow. 1994. *Mycobacterium marinum* persists in cultured mammalian cells in a temperature-restricted fashion. Infection and Immunity 62:3222-3229.

Reichenbach-Klinke, H.H. 1972. Some aspects of mycobacterial infections in fish. Symposium of the Zoological Society of London No. 30, London, England.

Sakanari, J.A., C.A. Reilly, and M. Moser. 1983. Tubercular lesions in Pacific coast populations of striped bass. Transactions of the American Fisheries Society 112:565-566.

Schreck, C.B. 1996. Immunomodulation: Endogenous factors. Pages 311-337 *in*: G. Iwama and T. Nakanishi, editors. The fish immune system: Organism, pathogen and environment. Academic Press, New York, New York, USA.

Smith, A. 1996. Mycobacteriosis in intensively cultured fish. Aquacultural Engineering Society Proceedings II: Successes and failures in commercial recirculating aquaculture 1:28-31.

Stevens, D.E., D.W. Kohlhorst, L.W. Miller, and D.W. Kelley. 1985. The decline of striped bass in the Sacramento-San Joaquin Estuary, California. Transactions of the American Fisheries Society 114:12-30.

Swanson, C., R.C. Mager, S.I. Doroshov, and J.J. Cech Jr. 1996. Use of salts, anesthetics, and polymers to minimize handling and transport mortality in delta smelt. Transactions of the American Fisheries Society 125:326-332.

Sweetnam, D.A. 1999. Status of delta smelt in the Sacramento-San Joaquin Estuary. California Fish and Game 85:22-27.

Wayne, L.G. and G.P. Kubica. 1986. The mycobacteria. Pages 1435-1457 *in*: P. Sneath, N. Mair, M. Sharpe, and J. Holt, editors. Bergey's manual of systematic bacteriology, volume 2. Williams & Wilkins Co., Baltimore, Maryland, USA.

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A LIST OF FRESHWATER, ANADROMOUS, AND EURYHALINE FISHES OF CALIFORNIA

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The freshwater fishes of California include 67 native resident or anadromous species, 53 non-native species, and 5 marine species that occur in fresh water, for a total of 125 species. Within these species, 100 taxa at lower levels (subspecies, Evolutionary Significant Units, runs of anadromous fish) are recognized. The number of freshwater fish species in California is increasing due to invasions of non-native fishes, which are becoming established at a rate of about 1 species every 3 years. Of the native fishes, 5 full species are extinct in California. Thus, the actual number of species maintaining populations in the state is 120. Of extant native species, 15 (22%) are threatened with extinction in the near future. Only 27 native species (40%) can be regarded as having secure populations.

INTRODUCTION

The fishes of California's inland waters are a mixture of resident, anadromous, and euryhaline species, both native and non-native. The first attempt to compile a comprehensive list of these species was Evermann and Clark (1931), who listed 78 native species and 32 introduced species. Native species included an anomalous Pacific hagfish, *Epatretus stouti*, from the Eel River, 14 forms all now considered to be rainbow trout, and 18 other forms that are either no longer recognized as species or were mistakenly considered to be part of the California fish fauna. Of the 32 non-native species listed, 9 did not become established and brown trout were listed as 2 species. Thus only 67 (45 native, 22 non-native) of the 120 species of Evermann and Clark would be recognized in the fauna today. However, many of their species are now recognized as subspecies, a concept not in wide use by ichthyologists of that era.

The next official list (Shapovalov and Dill 1950) included 101 species (64 native, 26 non-native, and 11 euryhaline marine), 89 of which are on the present list (although not necessarily by the same common or scientific names). This list also included

47 subspecies. Of the 12 species no longer recognized: 9 are now listed as subspecies, 1 (bigmouth buffalo, *Ictiobus cyprinellus*) is a non-native species no longer found in California, 1 (woundfin, *Plagopterus argentissimus*) is based on a questionable record, and 1 is a marine species (surf smelt, *Hypomesus pretiosus*) that, to our knowledge, has never been found in fresh water. This list was expanded in 1959 (Shapovalov et al. 1959) to include 110 species (64 native, 32 non-native, 14 euryhaline marine), 100 of which are recognized in the present list; 45 subspecies were also listed. Moyle (1976) largely followed this list, but his total was 130 species (81 native, 49 non-native), reflecting taxonomic changes and new introductions. Hubbs et al. (1979) published a list of all California fishes and included about 120 freshwater species (90 native, 30 non-native). A number of their native forms were undescribed species listed without explanation. The revised list of Shapovalov et al. (1981) included 124 species (66 native, 45 non-native, 13 euryhaline marine), with 58 subspecies listed as well. Mentioned in these lists, but not included in the total for freshwater species, were 4 marine species introduced into the Salton Sea: sargo, *Anisotremus davidsoni*; gulf croaker, *Bairdiella icistia*; orangemouth corvina, *Cynoscion xanthulus*; and shortfin corvina, *C. parvipinnis*. The shortfin corvina apparently is no longer present in the sea, whereas the other 3 species are being stressed by increasing salinities (S. Keeney, California Department of Fish and Game [CDFG], personal communication). These species are not included on this list as well.

The following list is essentially an updated version of Shapovalov et al. (1981), taking into account new information on southern California fishes presented in Swift et al. (1993), on introduced fishes by Dill and Cordone (1997), and on all fishes by Moyle (2001). The order of families and species reflect evolutionary relationships as described in Moyle and Cech (2000) and Moyle (2001). The purposes of this list are to 1) provide a handy checklist for freshwater, anadromous, and euryhaline fishes likely to be found in the inland waters of California; 2) promote uniformity in both common and scientific names, especially for subspecies and other categories below the species level; and 3) summarize current status and legal protection afforded each species. The names for species follow that of the American Fisheries Society (Robins et al. 1991), while the names for subspecies follow Moyle (2001) in describing geographic regions in which each form is found. Discussions of the taxonomy of each species, which are the basis for the names, can be found in Moyle (2001). At the species level, only forms that have been formally recognized as species are included; at the subspecies level, forms that are likely to be formally described in the future are included along with described forms. The following forms are included in the list:

1. native species that have reproducing populations in California,
2. native species once found in the state that are now extinct,
3. euryhaline marine species regularly collected in fresh water (defined as <3‰ salinity),
4. non-native species with reproducing populations in the state,
5. subspecies recognized in Moyle (2001), and

6. Evolutionary Significant Units (ESUs) recognized by the National Marine Fisheries Service (NMFS) for anadromous salmonids.

Excluded from this list are:

1. non-native species that do not have populations established in California as of 2000 and non-native species that once had breeding populations in the state but no longer exist here, and
2. marine species for which there are no or questionable records for prolonged or repeated presence in water with salinity of <3‰.

Numerous marine species occur in estuaries at moderate salinities, but do not occur, except by accident, in fresh water. Swift et al. (1993), for example, list 23 species that have been collected in estuaries of southern California, only 11 of which are included in this list.

CHANGES TO THE LIST

The following are changes to the list compiled by Shapovalov et al. (1981) reflected in the present list.

Petromyzontidae

Our understanding of the complex of lampreys in the Klamath basin is still confused, but the best evidence indicates that the Modoc brook lamprey, *Lampetra folletti*, of the 1981 list is not a valid species, whereas the Klamath River lamprey should be added to the list (Moyle 2001). The Pacific brook lamprey, *L. pacifica*, of the 1981 list appears to be similar enough to the western brook lamprey to be treated as the same species for now, although the western brook lamprey probably represents multiple species (Moyle 2001). The Goose Lake lamprey of the present list is probably a distinct species, but pending clarification of its status, it is treated as an undescribed subspecies of the Pacific lamprey.

Acipenseridae

The 1981 list calls the green sturgeon a subspecies, *A. medirostris medirostris*, with presumably the other subspecies being the Asian green sturgeon, *A. m. mikadoi*. Best evidence indicates that the 2 forms are separate species, so the subspecies name is no longer needed (Moyle 2001).

Clupeidae

The Pacific herring is now regarded as a species rather than as a subspecies of *Clupea harengeus*, as indicated in the 1981 list. It is common in estuaries but rarely occurs in water of low salinity so is marginal as a species on this list.

Cyprinidae

The various undescribed subspecies of tui chub, California roach, and speckled

dace that are listed are the result of re-evaluation of the various geographic forms of the species by a number of researchers (Moyle 2001).

The woundfin, *Plagopterus argentissimus*, was on most early lists, but was removed without explanation by Shapovalov et al. (1959). W.L. Minckley (Arizona State University, personal communication) indicates it could be regarded as an extinct member of the California fauna because it occurs in the Gila River, Arizona, and in other Colorado River tributaries in upstream states. Thus, a short section of the Colorado River that borders California is within the historic range of woundfin. However, in the absence of California records, we leave it off the list.

Catostomidae

The flannelmouth sucker is added to the list as an extinct native species because historic and archaeological records indicate it was once present in the lower Colorado River. In recent years, it has become re-established below Davis Dam, Nevada-Arizona, just above the California reach of the river (G. Mueller, U.S. Geological Survey, Denver, Colorado, USA, personal communication).

Esocidae

The northern pike was illegally introduced and has established a population in Davis Lake (Plumas County), a reservoir on Grizzly Creek, a tributary of the Feather River. As of 2000, efforts to eradicate the population had failed.

Osmeridae

The surf smelt, *Hypomesus pretiosus*, is removed from the list because it is primarily a marine species and does not occur in water <10‰.

Salmonidae

Salmon and Steelhead

Chinook salmon, coho salmon, and steelhead are subdivided into the units widely recognized for management by state and federal agencies, with each unit recognizable by a combination of genetic, ecological, and life history traits. Evolutionary Significant Units were developed by NMFS for the purpose of defining distinct population segments under the Endangered Species Act of 1973. "An ESU is a population (or group of populations) that is 1. substantially reproductively isolated from other conspecific population units and 2. represents an important component in the evolutionary legacy of the species" (Waples 1991:12). For chinook salmon, beneath each ESU, we have listed the runs that are or were native to each river system because they are recognized as distinct management units, presumably with a genetic basis. These runs are more geographically isolated from one another than are stream-specific runs of coho salmon, steelhead, or coastal cutthroat trout. For steelhead, aside from ESUs, we only list summer steelhead. Whereas summer

steelhead are genetically very close (but not identical) to winter-run steelhead in the same regions, their life history and distribution patterns are so distinct they merit recognition as separate taxa. For coastal cutthroat trout, only 1 ESU is recognized by NMFS for southern Oregon and northern California, so the ESU is not listed as such (Johnson et al.[1] 1999).

Golden Trout

In previous lists, golden trout have been treated as a separate species from rainbow trout, a distinction that is not justified genetically (Behnke 1992), although the 3 recognized subspecies of golden-type trout found in the upper Kern River basin are placed together in our list. Likewise, the 3 recognized forms of redband-type trout from the upper Sacramento River and Eagle Lake drainages are placed together because of geographically based relationships. Resident rainbow trout found throughout California, but not in the golden or redband trout clusters, are either offshoots of regional steelhead populations (and can be considered part of the ESU as a consequence) or are the result of planting fish of various origins. Kamloops rainbow trout are listed mainly to symbolize the widespread rearing and planting of rainbow trout strains that originated from other regions of western North America.

Dolly Varden

Shapovalov et al. (1981) include Dolly Varden, *Salvelinus malma*, on their list based on an 1877 record from the McCloud River, even though they expressed skepticism about the validity of the record. We agree with their skepticism and remove the species from the list.

Poeciliidae

The western mosquitofish, recognized as a subspecies in the 1981 list, is now recognized as a full species (Wooten et al. 1988). As far as is known, the eastern mosquitofish, *Gambusia holbrooki*, has not been successfully introduced into California.

Gasterosteidae

The brook stickleback was introduced into the Scott River (Siskiyou County), tributary to the Klamath River, around 1990 and is well established there (Moyle 2001). The unarmored and resident threespine sticklebacks represent multiple evolutionary descents from anadromous threespine sticklebacks, so are not really geographic subspecies (Moyle 2001). The names of the 3 forms are retained here for convenience until stickleback ESUs are recognized as they are for salmon. The Shay Creek stickleback, on the other hand, is a distinctive form isolated at a single locality

[1] Johnson, O.W. and 7 others. 1999. Status review of coastal cutthroat trout from Washington, Oregon, and California. NOAA Technical Memorandum NMFS-NWFSC-37.

in the San Bernardino Mountains and may deserve species status.

Syngnathidae

Shapovalov et al. (1981) added bay pipefish, *Syngnathus leptorhynchus*, to the list without explanation, but presumably because Moyle (1976) reported pipefish at salinities as low as 9‰. However, occurrences outside of marine eel grass beds are rare and there seems little reason to consider pipefish part of California's freshwater fish fauna.

Cottidae

The sharpnose sculpin, *Clinocottus acuticeps*, is omitted from the list because its previous inclusion was based on a single collection from "freshwater rills on the beach at Crescent City, Del Norte County" (Shapovalov et al. 1959:168). There is no indication it regularly occurs in the lower reaches of coastal streams. The 3 undescribed subspecies of prickly sculpin are listed because they seem to be distinctive in distribution and ecology (Moyle 2001). The 3 subspecies of marbled sculpin are added based on Daniels and Moyle (1984).

Centrarchidae

Shapovalov et al. (1959) list 2 subspecies each of bluegill, largemouth bass, and spotted bass. We omit them here because there seems little point in identifying subspecies introduced outside their normal geographic range that are presumably freely hybridizing with one another.

Cichlidae

The 3 *Oreochromis* species of tilapia are included because there is reasonable evidence of their establishment in southern California. However, the forms present bear little resemblance to their wild ancestors, presumably as a result of genetic bottlenecks during the introduction process and hybridization with each other (Costa-Pierce and Doyle 1997, Dill and Cordone 1997). Costa-Pierce and Doyle (1997) found that Nile and Mozambique tilapia were so different from their African ancestors that they recommended calling them regional strains labeled "California Nile tilapia" and "California Mozambique tilapia."

Gobiidae

Three species of *Tridentiger* have been introduced into California and 2 have been added to the list. The shimofuri goby lives and breeds in freshwater (Matern and Fleming 1995). Records of chameleon goby, *T. trigonocephalus*, in fresh water are undoubtedly this species because chameleon goby are largely marine. The shokihaze goby is a recent (ca. 1997) invader of the San Francisco Bay Estuary, where it is apparently established, living in water ranging from fresh to brackish

(K.A. Hieb, CDFG, personal communication).

STATUS

The native freshwater fishes of California are in a general state of decline (Moyle and Williams 1990) and many species have been given special status designations as a result. Fish found in California have been classified as threatened or endangered by the California Fish and Game Commission (state status) (CDFG[2] 2000a; California Fish and Game Code[3] 2000; California Code of Regulations, Title 14[4] 2000) or by the federal government under the Endangered Species Act of 1973 (Federal Register[5] 1996, USFWS[6] 1997). California Species of Special Concern is a CDFG designation (CDFG[7] 2000b) given to species that appear to be vulnerable to extinction because of declining populations, limited ranges, or continuing threats (Moyle et al.[8] 1995). California Protected and California Fully Protected fishes may not be taken or possessed without a permit (California Fish and Game Code[3] 2000; California Code of Regulations, Title 14[4] 2000). The following are the symbols used in the list for the various official status designations, along with the number of species in each category.

CSC	California Species of Special Concern	48 species
CCE	California Candidate Endangered	1 species
CT	California Threatened	4 species
CE	California Endangered	14 species
FT	Federal Threatened	12 species
FE	Federal Endangered	16 species

Because legal status does not always reflect actual status of a species, evaluations of status found (and justified) in Moyle (2001) are also included, for both native and introduced species, as follows:

Native Species

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[2] CDFG. 2000a. State and federally listed endangered and threatened animals of California. California Department of Fish and Game, Sacramento, California, USA.

[3] California Fish and Game Code. 2000. LawTech Publishing Company, Ltd., San Clemente, California, USA.

[4] California Code of Regulations, Title 14. 2000. California Department of Fish and Game, Sacramento, California. 95814.

[5] Federal Register. February 28, 1996. 61(40):7596-7613.

[6] USFWS. 1997. Endangered and threatened wildlife and plants. 50CFR17.11 ans 17.12, October 31, 1997. USDI Fish and Wildlife Service, Washington, D.C., USA.

[7] CDFG. 2000b. Special animals. California Department of Fish and Game, Sacramento, California, USA.

[8] Moyle, P.B., R.M. Yoshiyama, J.E. Williams, and E.D. Wikramanayake. 1995. Fish species of special concern in California, 2nd edition. California Department of Fish and Game, Sacramento,California, USA.

N1 Extinct. The species is extirpated from California; some species are globally extinct.

N2 Threatened or endangered. The species is likely to become extinct in the near future (<50 years) unless steps are taken to save it. An endangered species is on a more rapid path to extinction than a threatened species. Most of these species are formally listed by either the state or federal government.

N3 Special concern. The species is in decline or has a very limited distribution, so special management is needed to keep it from becoming threatened or endangered.

N4 Watch list. The species appears to be declining but is not yet in serious trouble. Its populations need to be monitored to see if special protective action is necessary.

N5 Stable or increasing. The species is abundant or increasing in population.

EM Euryhaline marine species regularly, if uncommonly, found in water <3‰ salinity.

Introduced (Non-native) Species

I1 Extinct. The species was once established, but the introduction failed. No species in this category is included on this list.

I2 Small, highly localized populations. The species is established, but only in a handful of localities, and is stable or declining in numbers.

I3 Localized and likely to become more widespread or already widespread, but not abundant in most areas. Alternately, it may be fairly common, but is declining. The species is usually a recent introduction and is just starting to expand its range or is a long-established species that tends to be only locally abundant.

I4 Widespread and stable. The species is widely distributed, but seems to have reached the limits of its range. Presumably it is integrated into local ecosystems.

I5 Widespread and expanding. The species is still expanding its range to all suitable habitats in the state.

THE LIST

PETROMYZONTIDAE (lampreys)	
river lamprey, *Lampetra ayersii*	N4, CSC
Kern brook lamprey, *Lampetra hubbsi*	N3, CSC
western brook lamprey, *Lampetra richardsoni*	N4
Pacific lamprey, *Lampetra tridentata*	
coastal Pacific lamprey, *L. t. tridentata*	N4
Goose Lake lamprey, *L. t.* ssp.	N3, CSC
Pit-Klamath brook lamprey, *Lampetra lethophaga*	N5
Klamath River lamprey, *Lampetra similis*	N4, CSC
ACIPENSERIDAE (sturgeons)	

green sturgeon, *Acipenser medirostris*	N3, CSC
white sturgeon, *Acipenser transmontanus*	N5
ELOPIDAE (tenpounders)	
machete, *Elops affinis*	EM
CLUPEIDAE (herrings)	
Pacific herring, *Clupea pallasi*	EM
threadfin shad, *Dorosoma petenense*	I4
American shad, *Alosa sapidissima*	I3
CYPRINIDAE (minnows)	
tui chub, *Gila bicolor*	
Mohave tui chub, *G. b. mohavensis*	N2, CE, FE
Lahontan creek tui chub, *G. b. obesa*	N5
Lahontan lake tui chub, *G. b. pectinifer*	N3, CSC
Owens tui chub, *G. b. snyderi*	N2, CE, FE
Goose Lake tui chub, *G. b. thalassina*	N3, CSC
Cowhead Lake tui chub, *G. b. vaccaceps*	N2, CSC
Eagle Lake tui chub, *G. b.* ssp.	N4, CSC
High Rock Spring tui chub, *G. b.* ssp.	N1, CSC
Pit tui chub, *G. b.* ssp.	N5
Klamath tui chub, *G. b. bicolor*	N5
blue chub, *Gila coerulea*	N3
Thicktail chub, *Gila crassicauda*	N1
bonytail chub, *Gila elegans*	N1, CE, FE
arroyo chub, *Gila orcutti*	N3, CSC
hitch, *Lavinia exilicauda*	
Clear Lake hitch, *L. e. chi*	N3, CSC
Sacramento hitch, *L. e. exilicauda*	N4
Monterey hitch, *L. e. harengus*	N4
California roach, *Lavinia symmetricus*	
Sacramento roach, *L. s. symmetricus*	N4
San Joaquin roach, *L. s.* ssp.	N3, CSC
Red Hills roach, *L. s.* ssp.	N2
Tomales roach, *L. s.* ssp.	N4, CSC
Pit roach, *L. s. mitrulus*	N3, CSC
Navarro roach, *L. s. navarroensis*	N5, CSC
Gualala roach, *L. s. parvipinnis*	N4, CSC
Monterey roach, *L. s. subditus*	N4
Sacramento blackfish, *Orthodon microlepidotus*	N4
Clear Lake splittail, *Pogonichthys ciscoides*	N1
Sacramento splittail, *Pogonichthys macrolepidotus*	N3, CSC, FT
hardhead, *Mylopharodon conocephalus*	N4, CSC
Sacramento pikeminnow, *Ptychocheilus grandis*	N5
Colorado pikeminnow, *Ptychocheilus lucius*	N1, CE, FE
speckled dace, *Rhinichthys osculus*	

Klamath speckled dace, *R. o. klamathensis*	N5
Sacramento speckled dace, *R. o.* ssp.	N5
Lahontan speckled dace, *R. o. robustus*	N5
Santa Ana speckled dace, *R. o.* ssp.	N2, CSC
Amargosa speckled dace, *R. o. nevadensis*	N2, CSC
Owens speckled dace, *R. o.* ssp.	N2, CSC
Long Valley speckled dace, *R. o.* ssp.	N2
Lahontan redside, *Richardsonius egregius*	N5
golden shiner, *Notemigonus crysoleucas*	I5
red shiner, *Cyprinella lutrensis*	I5
fathead minnow, *Pimephales promelas*	I5
tench, *Tinca tinca*	I2
goldfish, *Carassius auratus*	I4
common carp, *Cyprinus carpio*	I4
grass carp, *Ctenopharyngodon idella*	I3
CATOSTOMIDAE (suckers)	
mountain sucker, *Catostomus platyrhynchus*	N3, CSC
Santa Ana sucker, *Catostomus santaanae*	N2, CSC, FT
Sacramento sucker, *Catostomus occidentalis*	
Humboldt sucker, *C. o. humboldtianus*	N4
Goose Lake sucker, *C. o. lacusanserinus*	N3, CSC
Monterey sucker, *C. o. mniotiltus*	N5
Sacramento sucker, *C. o. occidentalis*	N5
Klamath largescale sucker, *Catostomus snyderi*	N3, CSC
Tahoe sucker, *Catostomus tahoensis*	N5
Owens sucker, *Catostomus fumeiventris*	N4, CSC
flannelmouth sucker, *Catostomus latipinnis*	N1
Modoc sucker, *Catostomus microps*	N2, CE, FE
Klamath smallscale sucker, *Catostomus rimiculus*	N5
shortnose sucker, *Chasmistes brevirostris*	N2, CE, FE
Lost River sucker, *Deltistes luxatus*	N2, CE, FE
razorback sucker, *Xyrauchen texanus*	N2, CE, FE
COBITIDAE (loaches)	
oriental weatherfish, *Misgurnus anguillicaudatus*	I2
ICTALURIDAE (bullhead catfishes)	
white catfish, *Ameiurus catus*	I4
black bullhead, *Ameiurus melas*	I5
yellow bullhead, *Ameiurus natalis*	I3
brown bullhead, *Ameiurus nebulosus*	I4
blue catfish, *Ictalurus furcatus*	I3
channel catfish, *Ictalurus punctatus*	I4
flathead catfish, *Pylodictis olivaris*	I3
ESOCIDAE (pikes)	
northern pike, *Esox lucius*	I3

OSMERIDAE (smelts)	
wakasagi, *Hypomesus nipponensis*	I5
delta smelt, *Hypomesus transpacificus*	N2, CT, FT
longfin smelt, *Spirinchus thaleichthys*	N3, CSC
eulachon, *Thaleichthys pacificus*	N3, CSC
SALMONIDAE (salmon and trout)	
mountain whitefish, *Prosopium williamsoni*	N4
coho salmon, *Oncorhynchus kisutch*	
northern California/southern Oregon ESU	N2, CSC, FT
central California ESU	N2, CE, FT
chinook salmon, *Oncorhynchus tshawytscha*	
California coastal ESU	FT
Smith River fall run	N4
lower Klamath fall run	N3
Redwood Creek fall run	N2
Little River fall run	N2
Mad River fall run	N2
Bear River fall run	N2
Mattole River fall run	N2
Humboldt Bay tributaries fall run	N2
Eel River fall run	N2
Russian River fall run	N1
upper Klamath and Trinity rivers ESU	
upper Klamath-Trinity fall run	N3
Klamath-Trinity spring run	N2, CCE
Klamath late fall run	N1
Central Valley fall run ESU	N4
Sacramento late fall run ESU	N2
Sacramento spring run ESU	N2, CT, FT
Sacramento winter run ESU	N2, CE, FE
pink salmon, *Oncorhynchus gorbuscha*	N1, CSC
chum salmon, *Oncorhynchus keta*	N2, CSC
sockeye salmon, *Oncorhynchus nerka*	
anadromous form	EM
kokanee	I4
rainbow trout, *Oncorhynchus mykiss*	
coastal rainbow trout, *O. m. irideus*	
Klamath Mountains Province steelhead ESU	N4
Klamath winter-run steelhead ESU	N4
Klamath summer steelhead	N3, CSC
northern California steelhead ESU	
north coast winter-run steelhead	N3, FT
north coast summer steelhead	N2, CSC, FT
Central Valley steelhead ESU	N2, FT

central coast steelhead ESU	N4, FT
south/central coast steelhead ESU	N2, FT
southern steelhead ESU	N2, FE
upper Kern rainbow trout	
California golden trout, *O. m. aguabonita*	N2, CSC
Little Kern golden trout, *O. m. whitei*	N2, FE
Kern River rainbow trout, *O. m. gilberti*	N2, CSC
upper Sacramento redband trout	
McCloud River redband trout, *O. m. stonei*	N2, CSC
Goose Lake redband trout, *O. m.* ssp.	N2, CSC
Eagle Lake rainbow trout, *O. m. aquilarum*	N2, CSC
Kamloops rainbow trout, *O. m. "gairdneri"*	I4
cutthroat trout, *Oncorhynchus clarkii*	
coast cutthroat trout, *O. c. clarkii*	N3, CSC
Lahontan cutthroat trout, *O. c. henshawi*	N2, FT
Paiute cutthroat trout, *O. c. seleniris*	N2, FE
Colorado River cutthroat trout, *O. c. pleuriticus*	I2
brown trout, *Salmo trutta*	I5
bull trout, *Salvelinus confluentus*	N1, CE
brook trout, *Salvelinus fontinalis*	I4
lake trout, *Salvelinus namaycush*	I4
CYPRINODONTIDAE (pupfishes)	
desert pupfish, *Cyprinodon macularius*	N2, CE, FE
Amargosa pupfish, *Cyprinodon nevadensis*	
Amargosa pupfish, *C. n. amargosae*	N2, CSC
Tecopa pupfish, *C. n. calidae*	N1
Saratoga Springs pupfish, *C. n. nevadensis*	N2, CSC
Shoshone pupfish, *C. n. shoshone*	N2, CSC
Owens pupfish, *Cyprinodon radiosus*	N2, CE, FE
Salt Creek pupfish, *Cyprinodon salinus*	
Cottonball Marsh pupfish, *C. s. milleri*	N2, CT
Salt Creek pupfish, *C. s. salinus*	N2, CSC
FUNDULIDAE (killifishes)	
California killifish, *Fundulus parvipinnis*	N5
rainwater killifish, *Lucania parva*	I4
POECILIIDAE (livebearers)	
western mosquitofish, *Gambusia affinis*	I5
sailfin molly, *Poecilia latipinna*	I3
shortfin molly, *Poecilia mexicana*	I2
porthole livebearer, *Poeciliopsis gracilis*	I2
ATHERINOPSIDAE (silversides)	
topsmelt, *Atherinops affinis*	EM
inland silverside, *Menidia beryllina*	I5

GASTEROSTEIDAE (sticklebacks)	
brook stickleback, *Culaea inconstans*	I3
threespine stickleback, *Gasterosteus aculeatus*	
anadromous threespine stickleback, *G. a. aculeatus*	N5
resident threespine stickleback, *G. a. microcephalus*	N5
unarmored threespine stickleback, *G. a. williamsoni*	N2, CE, FE
Shay Creek threespine stickleback, *G. a.* ssp.	N2, CSC
COTTIDAE (sculpins)	
Coast Range sculpin, *Cottus aleuticus*	N5
prickly sculpin, *Cottus asper*	
Central Valley prickly sculpin, *C. a.* ssp.	N5
Clear Lake prickly sculpin, *C. a.* ssp.	N4
coastal prickly sculpin, *C. a.* ssp.	N5
Paiute sculpin, *Cottus beldingi*	N5
riffle sculpin, *Cottus gulosus*	N5
Pit sculpin, *Cottus pitensis*	N5
marbled sculpin, *Cottus klamathensis*	
upper Klamath marbled sculpin, *C. k. klamathensis*	N5
lower Klamath marbled sculpin, *C. k. polyporus*	N5
bigeye marbled sculpin, *C. k. macrops*	N4, CSC
reticulate sculpin, *Cottus perplexus*	N4, CSC
rough sculpin, *Cottus asperrimus*	N4, CT
Pacific staghorn sculpin, *Leptocottus armatus*	
northern Pacific staghorn sculpin, *L. a. armatus*	N5
southern Pacific staghorn sculpin, *L. a. australis*	N5
MORONIDAE	
white bass, *Morone chrysops*	I4
striped bass, *Morone saxatilis*	I5
CENTRARCHIDAE (sunfish, bass, crappies, and relatives)	
Sacramento perch, *Archoplites interruptus*	N2, CSC
green sunfish, *Lepomis cyanellus*	I5
pumpkinseed, *Lepomis gibbosus*	I5
warmouth, *Lepomis gulosus*	I3
bluegill, *Lepomis macrochirus*	I4
redear sunfish, *Lepomis microlophus*	I4
redeye bass, *Micropterus coosae*	I3
smallmouth bass, *Micropterus dolomieu*	I4
spotted bass, *Micropterus punctulatus*	I5
largemouth bass, *Micropterus salmoides*	I5
white crappie, *Pomoxis annularis*	I4
black crappie, *Pomoxis nigromaculatus*	I4
PERCIDAE (perches)	
yellow perch, *Perca flavescens*	I3
bigscale logperch, *Percina macrolepida*	I5

EMBIOTOCIDAE (surfperch)	
shiner perch, *Cymatogaster aggregata*	EM
tule perch, *Hysterocarpus traski*	
Clear Lake tule perch, *H. t. lagunae*	N4
Russian River tule perch, *H. t. pomo*	N3, CSC
Sacramento tule perch, *H. t. traski*	N5
CICHLIDAE (cichlids)	
Mozambique tilapia, *Oreochromis mossambicus*	I5
blue tilapia, *Oreochromis aurea*	I3
Nile tilapia, *Oreochromis nilotica*	I3
redbelly tilapia, *Tilapia zilli*	I3
MUGILIDAE (gray mullets)	
striped mullet, *Mugil cephalus*	N5
GOBIIDAE (gobies)	
yellowfin goby, *Acanthogobius flavimanus*	I3
arrow goby, *Clevelandia ios*	EM
tidewater goby, *Eucyclogobius newberryi*	N2, CSC, FE
longjaw mudsucker, *Gillichthys mirabilis*	N5
shimofuri goby, *Tridentiger bifasciatus*	I5
shokihazi goby, *Tridentiger barbatus*	I3
PLEURONECTIDAE (righteye flounders)	
starry flounder, *Platichthys stellatus*	N5

CONCLUSIONS

The present list of freshwater, anadromous, and euryhaline marine fishes of California includes 67 native resident or anadromous species, 53 non-native species, and 5 marine species that occur in freshwater, for a total of 125 species. In addition, the list contains 100 taxa at lower levels (subspecies, ESUs, runs of anadromous fish). In 1959, the number of native resident or anadromous species recognized was 64, while the number of non-native species was 32. In 1981, the numbers were 66 and 45, respectively, indicating in the past 20 years non-native fishes have become established in California at a rate of about 1 species every 3 years. At least 30 non-native species are widespread in the state (categories I4 or I5). Of the native fishes, 5 full species are extinct, as are 4 lower taxa. Thus, the fish fauna numbers 120 extant species. Among the extant native species, 15 (22%) are threatened with extinction in the near future (N2). Only 25 (40%) of the native resident or anadromous species can be regarded as being secure (N5). Clearly, the freshwater fish fauna of California is continuing to change as native fishes become less abundant and diverse, while non-native fishes increase in species and numbers.

LITERATURE CITED

Behnke, R.J. 1992. Native trout of western North America. Monograph 6, American Fisheries Society, Bethesda, Maryland, USA.

Costa-Pierce, B.A. and R.W. Doyle. 1997. Genetic identification and status of tilapia regional strains in southern California. Pages 1-17 *in*: B.A. Costa-Pierce and J.E. Rakocy, editors. Tilapia aquaculture in the Americas, volume 1. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Daniels, R.A. and P.B. Moyle. 1984. Geographic variation and a taxonomic reappraisal of the marbled sculpin, *Cottus klamathensis*. Copeia 1984:949-959.

Dill, W.A. and A.J. Cordone. 1997. History and status of introduced fishes in California, 1871–1996. California Department of Fish and Game Fish Bulletin 178.

Evermann, B.W. and H.W. Clark. 1931. A distributional list of the species of freshwater fishes known to occur in California. California Department of Fish and Game Fish Bulletin 35.

Hubbs, C.L., W.I. Follett, and L.J. Dempster. 1979. List of the fishes of California. Occasional Papers of the California Academy of Sciences 133.

Matern, S.A. and K.J. Fleming. 1995. Invasion of a third Asian goby, *Tridentiger bifasciatus*, into California. California Fish and Game 81:71-76.

Moyle, P.B. 1976. Inland fishes of California, 1st edition. University of California Press, Berkeley, California, USA.

Moyle, P.B. 2001 (In press). Inland fishes of California, 2nd edition. University of California Press, Berkeley, California, USA.

Moyle, P.B. and J.J. Cech Jr. 2000. Fishes: An introduction to ichthyology, 4th edition. Prentice-Hall, Upper Saddle River, New Jersey, USA.

Moyle, P.B. and J.E. Williams. 1990. Loss of biodiversity in the temperate zone: Decline of the native fish fauna of California. Conservation Biology 4:275-284.

Robins, C.R. and 6 others. 1991. A list of common and scientific names of fishes from the United States and Canada, 5th edition. Special Publication 20, American Fisheries Society, Bethesda, Maryland, USA.

Shapovalov, L., A.J. Cordone, and W.A. Dill. 1981. A list of the freshwater and anadromous fishes of California. California Fish and Game 67:4-38.

Shapovalov, L. and W.A. Dill. 1950. A check list of fresh-water and anadromous fishes of California. California Fish and Game 36:382-391

Shapovalov, L., W.A. Dill, and A.J. Cordone. 1959. A revised check list of fresh-water and anadromous fishes of California. California Fish and Game 45:159-180.

Swift, C.C., T.R. Haglund, M. Ruiz, and R.N. Fisher. 1993. The status and distribution of the freshwater fishes of southern California. Bulletin of Southern California Academy of Sciences 92:101-167.

Waples, R.S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of "species" under the Endangered Species Act. U.S. National Marine Fisheries Service Marine Fisheries Review 53:11-22.

Wooten, M.C., K.T. Scribner, and M.H. Smith. 1988. Genetic variability and systematics of *Gambusia* in the southeastern United States. Copeia 1988:283-289.

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DISCOVERY OF SHASTA SALAMANDERS IN ATYPICAL HABITAT

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The Shasta salamander, *Hydromantes shastae*, is a state-listed threatened species endemic to a small region of Shasta County north and northeast of Redding, California. Until recently, all documented Shasta salamander observations occurred within very specific physical habitats. All previously known sites, with the exception of 1 lava rock outcrop (Papenfuss and Cross[1] 1980), occurred within 200 m of limestone rock formations, although no surveys have been conducted beyond this distance (Olsen and Lewendal[2] 1999).

While conducting terrestrial mollusk survey work for the U.S. Forest Service during spring and fall 1999, we discovered 6 Shasta salamanders at 6 locations in areas considered atypical habitat. The terrestrial mollusk surveys are time-constrained level-of-effort searches consisting of 1-person-hour of search time per 4 ha of habitat and are conducted using two 20-minute "point searches" and one 20-minute "sample area." A point search consists of searching throughout the 4-ha survey area looking for terrestrial mollusks in appropriate microhabitats (e.g., duff, litter, down woody debris, rocks, talus). A sample area is an approximately 25-m^2 area within the 4-ha survey area, which encompasses several suitable microhabitats.

The Shasta salamander discovery site locations occurred within the Shasta Lake Ranger District in the Reynolds, Dead Horse, and Arbuckle Creek drainages, small tributaries to the Pit River arm of Shasta Lake (40°45'N, 122°09'W to 40°46'N, 122°06'W) (Fig. 1). These sites ranged from 2.4 to 6.4 km from the nearest limestone formations, located to the northeast at Brock Mountain and Grey Rocks.

Generally, habitats at the discovery sites were similar. However, each site had its own microhabitat characteristics. They were characterized as montane hardwood-conifer (Mayer and Laudenslayer 1988), dominated by an overstory of douglas fir, *Pseudotsuga menziesii*; canyon live oak, *Quercus chrysolepis*; and California black oak, *Q. kelloggii*. Understory vegetation consisted of sparse to moderate shrub and herbaceous growth including poison oak, *Toxicodendron*

[1] Papenfuss, T. and D. Cross. 1980. The status of the Shasta salamander (*Hydromantes shastae*) in the Mountain Gate and Cedar Creek areas of Shasta County. BLM Contract Report. Bureau of Land Management, Redding, California, USA.

[2] Olson, D.H. and P.C. Lewendal. 1999. Survey protocol for the Shasta salamander (*Hydromantes shastae*). Pages 65-101 *in*: D.H. Olson, editor. Survey protocols for amphibians under the survey and management provisions of the Northwest Forest Plan, Version 3.0, October 1999. USDA Forest Service R5/R6 and USDI Bureau of Land Management, Oregon, Washington, California, USA.

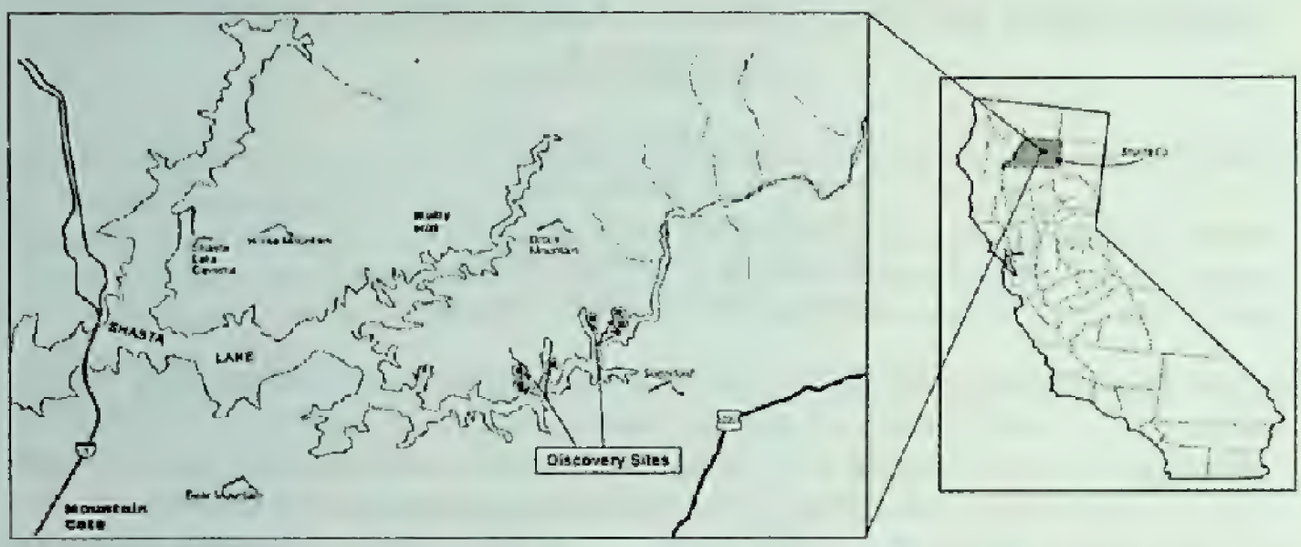

Figure 1. Shasta salamander, *Hydromantes shastae*, discovery site locations in atypical habitat, Shasta County, California.

diversilobum; snowdrop bush, *Styrax officinalis*; mock orange, *Philadelphus lewisii*; buckeye, *Aesculus californica*; five-finger fern, *Adiantum* sp.; sword fern, *Polystichum* sp., and various other annual grasses and forbs. Overstory canopy closure was dense, ranging from approximately 70 to 85%. Slopes at species discovery sites were steep (approximately 60–80%), with north, east, and northwest aspects. Terrestrial salamander microhabitats at these sites included moss, large and small woody debris, leaf litter, scattered rock, and occasional "rock-on-rock" (colluvium) pockets. No extensive talus slopes or other rocky sites occurred. Depending on the specific site, salamanders were found under down woody debris, rock/gravel, and leaf litter/duff. With the exception of 1 salamander found on rocky substrate, all salamanders were found on litter/duff substrate (i.e., the forest floor). The geologic formations within the discovery sites were all meta-sedimentary materials. No salamanders were measured; however, snout-vent length was estimated for all individual salamanders and ranged between 50 and 60 mm.

These discoveries may represent either populations isolated by the creation of Shasta Lake, or expanding populations from nearby limestone areas. These discoveries also demonstrate that within the known species range, Shasta salamanders occur in forested habitats not necessarily associated with limestone formations. Lack of prior observations of Shasta salamanders in these habitats may simply be due to a lack of survey effort resulting from assumptions regarding species habitat requirements.

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LITERATURE CITED

Mayer, K. E. and William F. Laudenslayer Jr., editors. 1988. A guide to wildlife habitats of California. California Department of Forestry and Fire Protection, Sacramento, California, USA.

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